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"A CONTRIBUTION to the STUDY of ANAPHYLAXIS".

by

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HISTOLOGICAL ANAPHYLAXIS C O N T E N T S.

	PAGE.
Preface.	1.
Introduction	3.
Symptoms in Anaphylactic Rabbits	5.
The Precipitin	16.
Precipitin Parallel to Severity of Symptoms.	20.
Changes in Precipitin during Anaphylactic Illness.	22.
Behaviour of the Rabbits Serum Complement.	26.
General Statement	36.
Connection between diminution of Complement and symptoms	39.
Bacteriolytic and Opsonic Effects.	43.
Delayed Blood Coagulation	56.
Behaviour of the Leucocytes	58.
The Time Relations.	62.
The Proteins producing Anaphylaxis	64.
The Control with Homologous Serum.	72.
Passive Transference.	73.
Serum Anaphylaxis in the Guineapig.	74.
Syptoms	76.
Blood Changes	78.
Proteins Employed.	82.
Passive Anaphylaxis in Guineapig	83.
Histological/	

	PAGE.
Histological Appearances.	86
Mechanism of the Symptoms	92
A. In the Rabbit.	
Cause of the Fall of Blood Pressure	95
The Respiratory Phenomena	97
B. In the Guineapig	100
Respiratory Exchange and Temperature.	101
The General Problem of Anaphylaxis	111
The Peptone Effect.	114
The Cause of Anaphylaxis	121
A. The Endotoxin theory	121
B. The Ferment Theory	124
C. Theories for the Immediate Anaphylactic Syndrome.	126
Besredka's Theory	129
Otto's Theory	132
Gay & Southard's Theory	133
D. Theory of Injury to the Endo- thelial Cells.	134
I. Changes in the fluids bathing the cells.	135
Alterations in Osmotic tension	135
" " Calcium Salts.	136.
II. Direct action on the Cell Protoplasm.	140
Chemistry of Anaphylaxis	147
The Serum Sickness in Man	148
Cause/	

	Page.
Cause	150.
Preventive measures.	153.
Other Practical applications.	154.
I. The Tuberculin Reaction	154.
II. Typhoid Diagnosis	156.
III. Cancer "	156.
IV. Food Idiosyncracies.	157.
Relation to Idiopathic Asthma	158.
" " Infectious Diseases	159.
Postscriptum.	160.

lar relation with certain organic poisons.

The unexpected nature of this reaction in the animal economy, - an increased disposition to suffer injury being teleologically so unlikely, gives it an intrinsic interest while the application of the conception to the processes in infective diseases (among others) may be of the greatest importance in clearing up the numerous obscurities in the natural history of these e.g. the period of incubation and the natural and acquired resistance.

On the special case of induced hypersusceptibility to horse-serum the widespread therapeutic employment of this as an antitoxic or antibacterial agent also conferred a considerable importance. It is with this form of anaphylaxis, experimentally/

experimentally induced, that this thesis is primarily concerned, but P R E F A C E. be made to take a general view of the condition as a response of a

The phenomenon of anaphylaxis has recently been the subject of extensive study both in America and the Continent. The impulse to this was chiefly the phenomenon of Theobald-Smith in the guineapig. Guineapigs treated with horse-serum at some previous date without showing symptoms, died rapidly on the horse-serum injection being repeated. Richet, working on pharmacological lines had observed a similar relation with certain organic poisons.

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experimentally induced, that this thesis is primarily concerned, but an attempt will be made to take a general view of the condition as a response of a well-defined character on the part of the organism to the introduction by unusual channels of beterologous organic matter.

The literature up to date bears almost entirely on the guineapig, the use of the rabbit in this research broadens the experimental basis for conclusions of a general nature.

INTRODUCTION.

In January 1909 at the suggestion of Dr. James Ritchie I injected intravenously two adult fresh rabbits with Horse Serum each 5 cc.; they displayed no symptoms either immediately or during the ten days succeeding. On the eleventh day Rabbit I., (1800 grams.) received $3\frac{1}{2}$ cc. of the same Horse-serum intravenous; the serum was warmed to $37^{\circ}\text{C}.$, and the injection, into the marginal vein of the ear, was completed in 40 seconds. Again no symptoms were produced. Rabbit II. (24008.) received with the same precautions 3 cc. of the same serum intravenous. During the injection it became restless, fell on its side as if paralysed and after one or two general convulsive movements and gasping respirations died in less than three minutes from commencing the injection; there could be no question of air-embolus and post mortem, no clot could be found in the heart or any organ. Two questions for solution emerged from this experiment.

What is the nature of this lethal action of Horse-serum, apparently innocuous ten days before, and/

and why should it, under apparently parallel conditions omit one animal and with a dose (per kilo. body weight) considerably smaller, destroy another. An answer to the latter question, it seemed likely, might give a clue to the former. Accordingly an investigation was begun on the effects of Horse Serum in rabbits and especially as to whether any differences in the blood serum of the animals corresponded with the degree of severity of the symptoms displayed.

The conditions in which symptoms were to be expected being known the investigation of their mechanism became possible. Deductions from the experiments were to be applied to the peculiar and occasionally dangerous effects of foreign serum known as serum-sickness in man, and to the general questions of immunity to foreign proteins including infective bacteria.

(The only records existing on the effects of Horse-serum on the rabbit were those of Arthus 1903 (2) in which the acquired susceptibility of rabbits to Horse-serum was first noted and a short statement of V. Pirquet and Shick 1905 (70) that this condition did not depend on the precipitin content of the rabbit's blood serum. Isolated observations of death occurring during administration of various proteins may be found scattered through the literature, probably the earliest being recorded by Magendie in 1839 cited by Morgenroth (60); a rabbit which had already received without/

without symptoms two injections of eggalbumin intravenous on being subjected several days after to a similar dose died immediately from its effects. Landois 1875 (52) records similar results in connection with his transfusion experiments, again however, without any attempt at a generalising explanation. The existence of a condition of increased susceptibility to certain poisons of a protein character as the result of a previous administration was first specially insisted upon by Ch. Richet (79) and was named by him 'anaphylaxis' ($\alpha\nu\alpha$ = contrary to $\phi\rho\lambda\alpha\sigma\iota\varsigma$ = protection). Behaviour of this kind on the part of the guinea pig towards Horse-serum had been thoroughly studied by several observers (see later) under the name of the Theobald-Smith Phenomenon and had become the foundation of several conflicting theories of complex and far reaching nature).

SYMPTOMS OBSERVED IN ANAPHYLACTIC RABBITS.

The following description of the symptoms is based on observations on over forty rabbits, many of which were made to pass through the ordeal of an anaphylactic shock repeatedly. The first administration of Horse Serum whether subcutaneous intraperitoneal or intravenous has never, even in large quantities produced any disturbance immediate or delayed: the animal remains lively and does not lose weight. The injection of the same serum intravenous in 'sensitised' rabbits on the contrary is followed by a very uniform and definite set of symptoms. By a sensitised rabbit, I mean one which has received within certain limits of time before, one/

one or more doses of the serum by any of the three parenteral channels. Within fifteen seconds of starting the injection, in fact usually before it is completed, there is a well-marked blush of the ears, the veins swelling up and even pulsating; this lasts for a period of from a second or two, only, to about a minute and is associated with alteration in the rhythm of the breathing, little pauses and shortened breaths. Frequently at this stage there is some restlessness, the animal pricks up its ears and hops about.

Following sharply on this fleeting hyperaemia comes pronounced anaemia of the ear the veins collapse, the ear becomes cold, and strong heat and friction fail to produce the normal reaction of congestion: at this stage it is impossible to bleed, the ear vein cut across yields not a drop of blood and even the technique of bleeding, with an electric bulb to induce continuous congestion (see Illustration) which in normal animals permits the abstraction of large quantities of blood with rapidity and ease, (40-50 c.c.), now fails entirely.

Along with this there is marked panting respiration differing from the natural rapid panting of the rabbit by its vigour and depth: the heart beats/
beats/

beats very much more rapidly and feebly becoming almost impossible to count. The animal is restless, seeking apparently a comfortable attitude and holding its head high with an anxious expression.

At the second or third minute muscular weakness appears: the animal lies prostrate with legs stretched out and head resting on the floor; (see Illustration): the breathing is heaving and laboured, the heart beat very rapid and feeble; no notice is taken of sounds and even very vigorous disturbance, shaking and flicking, elicits no movement in response; the eyes are half shut and the expression is one of deep sleep. The abdomen at this period presents a striking appearance; it is large and full but the deep heaving movements of the diaphragm and chest transmit little or no excursion to it; the outlines of the stomach and caecum show through and loose folds of the flaccid muscular wall seem to flow out from the side of the prostrate rabbit. The skeletal muscles generally have lost tone, the spine bends and the limbs hang limply when the body is lifted. The anal sphincter is relaxed and first solid then loose and liquid faecal matter is discharged: if the bladder happens to be full urine/ this period the temperature is markedly sub-

normal.

urine also is discharged.

It is still practically impossible to get blood from the ear; the drop that may be expressed is very dark and tarry-looking and coagulates slowly. In 8-10 minutes this stage of depression reaches a maximum and rapid death may occur. Lying on its side the rabbit begins to kick convulsively, the breathing ceases, to be replaced by loud intermittent gasps, the head is thrown back with nostrils wide and eyeballs protruding. There may be two or three of these convulsive seizures at intervals of a few seconds with severe spasms of the back muscles and gasping or screaming attempts at respiration, then the respiration ceases, the corneal reflex disappears the animal is dead. The heart usually continues to beat for several minutes then gradually slows and stops.

In non-fatal cases all degrees of severity may be observed from those where the only evidence of disturbance may be a transient panting and local anaemia of the ears with complete recovery in 5-10 minutes to those, where for 30-40 minutes the animal remains in a state of impenetrable coma, recovering gradually in the course of the following two hours. During this period the temperature is markedly sub-normal./

normal. Recovery may take place even from the convulsive stage (one recovery) but as a rule the onset of convulsions presages rapid death. The animals which recover may be severely ill yet an hour after comport themselves absolutely as normal animals; not infrequently however, there is some languidity and absence of appetite lasting for some hours and in critical cases even for more than a day.

VARIED POST-MORTEM APPEARANCES. SYMPTOMS.

In fatal cases the appearances post mortem are very characteristic, the cadaver is limp (rigor mortis is slow to appear) with prominent abdomen and an extreme degree of exophthalmos. If the thorax is opened immediately after death the heart may still be seen beating feebly, the right chambers both markedly distended with dark venous blood while the left ventricle is contracted and pale; the lungs are usually distinctly pale; there may rarely be spots of congestion, the blood is always fluid even many hours after death and no thrombosis was ever found.

In the abdomen the general extreme congestion is the striking feature; the liver is very large/

ten days before had been in 'extremis' with a small large and of a deep plum colour, the small intestine a rich red with closely interlacing and prominent venules; the large veins, portal and mesenteric are much distended, the spleen dark and swollen. Petechial haemorrhages are in many cases scattered over the omentum and small intestines, more rarely in the pancreas and pyloric end of the stomach; the cardiac end of the stomach is usually free from congestion.

VARIATIONS IN THE SEVERITY OF THE SYMPTOMS.

In a group of rabbits whose preliminary serum-treatment has been rigidly the same, the most marked variations occur in the severity of the symptoms produced on delivering the assaulting dose. Some may show no single symptom or nothing that to an ordinary observer at least would be classed as disturbance; others after illness lasting 5-30 minutes recover completely, one or two of the group may die acutely displaying the syndrome and post-mortem appearances just described. But even in an individual rabbit great differences are observed in the effects of the intravenous administration of serum when this is performed at regular intervals of 10-12 days; an animal which ten/

ten days before had been in 'extremis' with a small intravenous dose may now undergo the same ordeal with symptoms slight and evanescent to be again severely ill at some future injection; (this is quite apart from the temporary insusceptibility to be described as immediately succeeding the anaphylactic illness).

The/

The following protocols of experiments will best illustrate the point.

White ♂ 1720 gram.
(fresh rabbit).

Himalaya ♀ 1670 gram.
(fresh rabbit).

Feb.14. Intravenous injection 5 cc. old Diphtheria-Antitoxin. (Horse-serum)	Feb.14. Intravenous injection 5 cc. old Diphtheria-Antitoxin. (Horse-serum).
Feb.23. Bled 2 cc. from ear-vein.	Feb.23. Bled 2 cc. from ear-vein.
Feb.25. 10.30 a.m.bled 7 cc. from ear-vein Rectal 1° = 102.1°.	Feb. 25. 10.45 a.m.bled 7 cc. from ear-vein.
11.50 a.m. intravenous injection $2\frac{1}{4}$ cc. Antitoxin (warm)	3.40 p.m. intravenous injection $2\frac{1}{4}$ cc. (warm) Antitoxin.
11.51 a.m. uneasiness, rapid breathing:	
11.52 a.m. panting lying on side continuing thus till	3.41 p.m. slight restlessness and panting.
12 noon, attempted to draw blood from ear-vein without success.	
12.0 to 12.20 p.m. breathing rapid heaving character-abdomen; very limp, marked muscular weakness; contents of rectum discharged, loose and watery.	3.50 p.m. apparently well; bled easily from ear-vein 6 cc.

12.20/

12.20 p.m. Rect.
1° = 100.3° F.

blood normal colour
and appearance.

12.30 p.m. One or
two drops of very
dark tarry-looking
blood expressed
from ear-vein.

11.37 a.m. some
disturbance of
breathing

12.40 p.m. still
prostrate does not
respond to touch-
breathing.

12.5 no definite
symptoms have ap-
peared; bled 5 cc.
without difficulty

1.20 p.m. sitting
up, breathing
still a little
laboured; bled
easily from ear
6 cc.

4.50 p.m. no
further symptoms
have developed.

4.10 p.m. Now
quite lively;
intravenous in-
jection of 4 cc.
same antitoxin.

4.50 p.m. No
symptoms have
developed.

INTERVAL OF 20 DAYS.

Mar. 17. 10.5 a.m. bled
6 cc. from ear-
vein.

10.15 a.m. bled
5 cc. from ear-
vein.

10.35-10.36 a.m.
intravenous in-
jection 5 cc.
inactivated
horse-serum (2
days old) warm:

11.55 a.m. intra-
venous injection.
5 cc. inactivated
horse-serum 2 days
old.

10.37/

faintest colour of turbidity. (See later Protocols).

The/

10.37 a.m.
restlessness began followed by prostration, and panting respiration; symptoms practically as severe as on Feb. 25th.

11.37 a.m. some disturbance of breathing

12.5 no definite symptoms have appeared; bled 5 cc. without difficulty from ear-vein.

10.45 one or two drops dark tarry blood expressed with difficulty from ear-vein.

11.25 bled slowly from ear 5 cc. blood rather dark in colour.

12 noon. Practically recovered.

These two rabbits treated alike displayed thus a pronounced divergence in their behaviour to a repeated dose. The difference in their blood serum was equally marked. The animal which on each occasion was profoundly ill, had a serum rich in precipitin towards horse-serum; the animal which on each occasion escaped without definite symptoms had a serum which reacted to horse-serum with only the faintest degree of turbidity. (See later Protocols).

The/

The fact is, of course, well known that animals injected with foreign serum develop in their blood a specific antibody which on contact with its antigen is thrown down in the form of a flocculent precipitate. The antigen forms a very small part of this precipitate which contains chiefly the englobin fraction of the immune serum. (Welch and Chapman (104), Franceschelli (109)).

THE PRECIPITIN.

The precipitin present in the serum was estimated in capillary glass tubes of about 3mm. diameter, drawn to a point at the closed end. Horse-Serum (or other antigen) was diluted with normal saline solution (Sodium Chloride .85 p.c.) to provide dilutions of six different degrees, 1 in 10, 1 in 100, 1 in 500, 1 in 1000, 1 in 5000, 1 in 10,000. By means of a fine pipette .2 cc. of each of these was filled into a capillary tube filling it to a depth of about 3 ctm.; the same pipette was used for each, beginning with the dilution 1-10,000. With a fresh pipette .2 cc. of the rabbit serum to be examined was made to run down the side of the tube; it sinks to the bottom and displacing the diluted antigen gives at the junction a zone of mixture of about 2 centimetres in which turbidity appears in proportion to the amount of precipitation occurring. (This procedure is much more efficient than the usual 'layering' method in revealing the existence of precipitin and distinguishing differences of degree). Notes were made (a) immediately on addition (b) after five minutes (c) after 24 hours, all at room/tem.

room temperature. From these the precipitin strength was estimated and the serum classed as 'very strong precipitin', 'strong', 'moderate', 'weak', 'very weak', or 'trace precipitin'. The points determining the category were (a) the density of the turbidity, (b) the height of the dilution of antigen still producing opacity and (c) the quantity of actual flocculent precipitate present after 24 hours. These were not always strictly concordant; frequently a serum weak in precipitin, i.e., which produced less density and a smaller quantity of precipitate than another, would still show turbidity at a dilution which had no effect on the latter. This difficulty occurs chiefly in comparing the serum of animals differing widely in the time elapsed since the last injection of antigen; after 20-30 days the quantity of precipitable protein in the serum diminishes very rapidly; the small amount of precipitable protein remaining seems however, to retain its sensitiveness and responds still with the very high dilutions. For this reason the third point, the amount of actual precipitate after 24 hours was the one chiefly relied on. Even to this, however, there is an objection: there is with each individual serum a certain dilution of antigen,/

antigen, (varying from 1 in 5 to 1 in 200) with which there is an optimum degree of precipitation; dilutions stronger or weaker give less result. It is impossible with the quantities of serum available to find and compare for each serum its optimum precipitation point and it is for this reason that any attempt at an apparently more accurate comparison by measuring minutely the actual precipitate (as in Nuttall's method (108)), was abandoned. By using the large field of six dilutions first mentioned, close distinctions can readily be made among a number of different sera.

THE FOLLOWING IS THE RECORD OF A TYPICAL PRECIPITIN ESTIMATION.

I.		Horse Serum Dilm.	1-10	1-100	1-500	1-1,000	1-5,000	1-10,000.
Serum White O	(immediate.	marked tur-	turbidity.	faint	trace tur-	Nil.	Nil.
	(bidity.		turbidity.	bidity.		
	(5 minutes.	flocculi.	dense tur-	turbidity.	turbidity.	turbidity.	faint tur
	(bidity.				bidity.
(Clear, no opalescence).	(
	(5 minutes.						
	(large de-	large de-	deposit.	small de-	flocculi.	flocculi
	(24 hours.	posit.	posit.		posit.		

CATEGORY THUS - STRONG PRECIPITIN.

II.		Serum Himalya	Immediate.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.
(faintly opalescence).	(
	(5 minutes.	faint tur-	faint tur-	Nil.	Nil.	Nil.	Nil.	Nil.
	(bidity.	bidity.					
	(24 hours.	minute de-	flocculi.	minute flocculi	flocculi	very minute.	Nil.	Nil.

WEAK PRECIPITIN.

III.		Serum B. & W.	Immediate	turbidity.	Nil.	Nil.	Nil.	Nil.	Nil.
(clear, faintly H.C.-tinged).	(
	(5 minutes.	marked tur-	turbidity.	turbidity.	faint tur-	Nil.	Nil.	Nil.
	(bidity.			bidity.			
	(24 hours.	Deposit	Deposit	flocculi	flocculi	flocculi	minute flocculi.	

MODERATE PRECIPITIN, RATHER WEAK.

PRECIPITIN PARALLEL TO SEVERITY OF SYMPTOMS.

It soon became evident by repeated experiments of the kind recorded that a close relation existed between the amount of precipitin present in the serum at the time of an intravenous injection and the severity of the symptoms induced by that injection. Rabbits whose serum contained a large quantity of precipitable protein became profoundly ill or died as the result of the intravenous dose, those with little precipitin escaped with slight and transient disturbance.

The following table is an example of this; (it illustrates also the point that a relatively small dose of Horse-serum, .1 cc. produces no sensitisation: this is in striking contrast to the effect in guinea pigs as will be shown later).

RABBITS TREATED WITH HORSE SERUM.

GREY ♂ 2800G.

BLACK ♂ 2100G.

Date	Intravenous Dose	Symptom	Precipitin	Intravenous Dose	Symptoms	Precipitin.
March 19th.	0.1 cc. (= first dose)	none	-	0.1 cc. (first dose)	none	-
			20 Days	elapse.		
			noon	5 cc.	none	none
April 9.	5 cc.	none	22 Days	elapse.		
			weak			
May 2.	5 cc.	illness of moderate severity.	precipitin	5 cc.	none	merest trace.
			11 Days	elapse		
May 13.	5 cc.	very severe illness.	strong precipitin	5 cc.	very slight completely recovered in five minutes.	very weak precipitin.

The blood serum obtained after death or subsidence of symptoms contains now no trace of precipitin: this observation is of some importance as will shortly appear.

CHANGES IN THE PRECIPITIN CONTENT DURING THE rapidly to ANAPHYLACTIC ILLNESS.

The serum from blood obtained after symptoms have appeared contains no precipitin; all dilutions of the antigen now fail to produce turbidity though before injection, even high dilutions may have thrown down copious flocculi. This condition maintains itself for four days; on the fifth there is a critical abundant reappearance of precipitin and in the course of 24-48 hours the serum practically regains its original strength. The appearance of the precipitin coincides exactly with the disappearance of the antigen from the circulation. On the fourth day the antigen is still demonstrable in abundance in the serum - shown by its precipitating a specific precipitin serum, on the fifth it has disappeared.

These periods are definitely different from those in the fresh animal. After the first dose, horse-serum continues to circulate for six to seven days apparently undiminished; (detected by a stock precipitin serum v. horse); on the seventh or eighth day it has disappeared and the first/

first traces of precipitin are present to increase rapidly to a maximum during the succeeding three days.

An example of an experiment on this point is submitted.

Wed. Mar. 17. White ♂) each received Horse Serum 5 cc.
) 10 days before
) each bled 10 cc. at 10 a.m.
) each received 5 cc. Horse
 Russian ♀) serum intravenous at 12 noon:

White had severe symptoms.

Russian indefinite.

each bled 4 cc. at 2 p.m.

Himalaya ♀ (Fresh rabbit) bled 2 cc. then
 received 5 cc. Horse-serum
 intravenous = first dose - no
 symptoms.

PRECIPITIN ESTIMATION.

	Horse Serum 1-10	1-100	1-1000)	
White Serum.	mark-	mark-	preci-	Strong
	ed pre-	ed	pi-	
before in-	cipi-	pre-	tate.)	
jection.	tate	cipa-	tate.)	precipi-
		tate.)	tin.
White Serum.	Nil.	Nil.	Nil.)	No pre-
)	
after inec-)	
tion.)	cipitin.
Russian Serum.	trace	trace	trace)	trace
	ppt.)	
before inec-)	
tion.)	precipi-
)	tin.
Russian/				

Tues. Mar. 22. Russian Serum Nil. Nil. Nil.) No precipitin.
Himalaya bled after injection.

Presence of Horse-serum - trace
Himalaya Serum. Nil. Nil. Nil.) normal serum.

Thurs. Mar. 18. Presence of Horse Serum in circulating blood.

each bled 2 cc.: Serum diluted 1-10 and added to serum of White 'before injection' (which gives definite precipitate with Horse Serum 1 in 1,000).

White Russian Himalaya.
precipitate. precipitate. precipitate.

Horse Serum present with each.

Fri. Mar. 19. each bled again investigated as above - Horse Serum present apparently in same amount.

Sat. Mar. 20. each bled again. Horse Serum present with each, still no precipitin against Horse-serum.

Mon. Mar. 22. each bled again - diluted the serum now gives with the strong precipitin Serum:-

White. No precipitate

Russian No precipitate

Himalaya. Definite precipitate.

Serum contained Precipitin and Horse Serum:-

With Horse Serum 1-10 1-100 1-1,000

White abundant precipitate. marked precipitate. precipitate.) strong precipitin.

Russian precipitate. precipitate. turbidity.) weak precipitin.

Himalaya Nil. Nil. Nil.) No precipitin.

Tues. Mar. 22. Himalaya bled again:

Presence of Horse-serum - trace?

In the Presence of Precipitin - trace?

Wed. Mar. 23. Himalaya bled again:

No precipitate with Serum White (before injection)

i.e., No Horse-serum in circulation.

Precipitin v. horse-serum.

Horse-serum 1-10, 1-100, 1-1000.

Himalaya.	turbi-	turbidi-	faint)	very
	dity.	ty.	turbi-	weak
			dity)	pre-
				cipi-
				tin.

Sat. Mar. 26. Himalaya bled again.

Horse-serum 1-10, 1-100, 1-1000

Himalaya.	marked preci-	preci-	moder-
	preci-	pitrate	ate pre-
	pitrate		cipi-
			tin.

THE BEHAVIOUR OF THE RABBITS SERUM COMPLEMENT.

In the description by McGowan (55) of experiments on the effect of complement-fixing combinations on complement 'in vivo', it was noted that the injection of 'sensitised' ox blood corpuscles in immunised animals led to a drop in the complementing strength of the serum: this was not so in fresh animals. Similarly the latter escaped without symptoms (one exception) while the former were severely ill one dying within 5 minutes. Horse-serum in combination with precipitin forms 'in vitro' a strong complement-fixing agent, as was first shown by Gay (35) and Moreschi (59); it was thus probable that 'in vivo' a similar action might take place and depression of the complementing power occur. Accordingly the minimal lytic dose of the animal's fresh serum for a combination of washed ox blood corpuscles with inactivated (56°C) haemolytic immune serum was determined: the serum obtained from blood drawn immediately before the symptom-producing injection of Horse serum was compared with that drawn from the ear, as soon after it as possible (owing to the difficulty of bleeding described, often one/

one or two hours after) or in fatal cases from the heart post mortem.

The two samples were treated as far as possible alike but it was usually necessary to separate the soft non-retracting clot of the second bleeding artificially and to centrifuge in order to get the serum, while the first separated serum spontaneously; control showed that this procedure had no effect on the complement strength of the serum. The estimation was conducted as soon as possible after bleeding and getting the serum clear and as a rule on the same day as the experimental injection. The protocol of a typical experiment will best illustrate the method.

AUG. 10. White ⁺ 2030G received 10 cc. Horse-Serum
Intra venous. = first dose.

B. & W. ⁺ 0 1820G " " "

AUG. 23. 13 days from sensitizing dose.

White ⁺ 2000G bled from ear 3 cc. at 3.40 p.m.

at 4.52 p.m. intravenous injection $2\frac{1}{2}$ cc. Horse-Serum (of March 19th).

4.54 collapsed, unconscious.

4.59 convulsed, respiration stopped but began again.

45.2 convulsed again; respiration ceased died.

White ⁺ 0 Heart cut open at once and 20cc. blood pipetted off:

B. & W. ⁺ 0 1820G bled 3 cc. at 3.50 p.m.

at 5.20 p.m. received $2\frac{1}{2}$ cc. Horse-Serum (19. III) intravenous.

5.22 p.m. ears blanched: some panting:

5.26 p.m. recovered: ears became congested (symptoms slight).

5.28 p.m. bled easily from ear 4 cc.

AUG. 24.

All four samples of blood kept at room temperature overnight: blood 'after' in each case yielded clear serum only after artificial separation and centrifuging.

Order of addition to tube
from left to right.

COMPLEMENT ESTIMATION.

TUBE	COM- PLEMENT.	H.I.B.	D.I.D. 1-10	SA- LINE	OX COR- PUSCLES.	1% SUSPENSION.	PUT IN INCUBATOR AT 37°C. 10.25 a.m.
White ♀	1. .2 cc.	.025cc	(2½ M.H.D)	.8	1 cc.)	Read at 10.55 a.m. = 1½ hour. 1055 complete At 11.55 = 1½ hr complete.	
Serum	2. .15 "	"		.85	"	"	
'before'	3. .125 "	"		.875	"	nearly complete.	
clear	4. .10 "	"		.9	"	lysis present	small deposit
	5. .075 "	"		.925	"	slight lysis	deposit
White ♀	6. .8 cc.	.025cc.		.2	1 cc.)	10.55 No lysis. At 11.55 about ½ lysis.	
	7. .6 "	"		.4	"	"	At 1½ hrs. trace lysis.
	8. .5 "	"		.5	"	"	"
Serum	9. .4 "	"		.8	"	"	No lysis.
'after'	10. .2 "	"		.8	"	"	"
clear.	11. .1 "	"		.9	"	"	"
B. & W. ♂	1. .2 cc	.025		.3	1 cc	10.55 complete	At 11.55 complete
	2. .15 "	"		.85	"	"	= 1½ hrs. "
'before'	3. .125 "	"		.875	"	much lysis.	practically "
	4. .10 "	"		.9	"	lysis.	small deposit.
clear	5. .075 "	"		.925	"		

TUBE, COM- H.I.B. DILD. 1-10 SA- OX COR- 1% PUT IN INCUBATOR
 PLEMENT. LINE PUSCLES. SUSPENSION. AT 37°C. 10.25 a.m.

B. & W. ♂	6.	.06	.025 cc	.4	1 cc.	10.55 nearly complete.	complete.
'after'	7.	.04	"	.6	"	much lysis	"
'faintly	8.	.035	"	.75	"	little lysis	small deposit.
H.B.	9.	.1	"	.9	"	no lysis	"
stained.	10.	.075	"	.925	"	no lysis	large deposit.

M.C.D. White ♀ 'before' = .125 cc. 'after' = ? more than .8 cc.

" B. & W. ♂ 'before' = .125 cc. 'after' = .4 cc.

The serum in the fatal case was practically inactivated, the serum in the case with slight symptoms had the M.C.D. weakened to three times that before injection:

The following table which is selected from over fifty observations illustrates the constant appearance of this phenomenon and its association with symptoms more or less proportional in severity.

A. EXAMPLES WHERE ANAPHYLACTIC SHOCK WAS FATAL.

[Blood taken from heart just after death compared with that obtained before injection]

HISTORY		ASSAULTING DOSE & EFFECT.		Minimum Complementing Dose for the same Haemolytic combination.	
				BEFORE.	AFTER.
White Russian ♀ 2100g	5 cc. Horse Serum intravenous 11 days before.	2½ cc. Horse Serum intravenous: death in 4 minutes.	0.05 cc.	0.3 cc.	
Black ♂ 1950g.	7 injection Ox-Serum during preceding 3 months - last dose 4 cc 30 days before.	5 cc Ox Serum (old) intravenous: death after 1½ hours illness.	0.1 cc.	0.6 cc (hardly complete)	
Grey ♂ 2400g (splenectomised)	2 previous intravenous injections of Ox-Serum last 20 days before (4 cc.)	5 cc. Ox Serum inactivated intravenous - death in 9 minutes.	0.1 cc.	0.8 cc.	
Black ♂ 1900g	4 previous injections Human Serum, last 9 days before, (2 cc.)	4 cc. Human Serum (inactivated) - death in 4 minutes	0.15 cc.	practically completely inactivated.	

B. WHERE RECOVERY TOOK PLACE AFTER SYMPTOMS OF VARYING SEVERITY.

[Blood obtained from ear-vein as soon as this became possible i.e., on recovery commencing].

HISTORY	ASSAULTING DOSE & EFFECT.	M.C.D. before	M.C.D. after.
Black ♀ 1920g. received 17½ cc Ox-Serum in 4 doses during previous 3 weeks: strong precipitin.	2½ cc. inactivated Ox Serum. Symptoms extremely severe for 2 hours.	.05 cc.	.2 cc trace of lysis only (bled 40 mins. after in-) (jection, only that (quantity of serum could) (be got owing to diffi- (culty of bleeding. after 2½ hrs .25 cc.
" 30 days after above, trace precipitin only.	4 cc inactivated Ox Serum. No symptoms.	.15 cc.	.2 cc - difference not very marked.
B. & W. Rus. ♂ 1600g. fresh animal.	Horse Serum 5 cc. intravenous no symptoms.	.2 cc.	.2 cc.
" 10 days after, precipitin very slight.	3 cc Horse Serum intravenous. No symptoms.	.15 cc.	.2 cc. (bled 10 minutes after injection)

HISTORY		ASSAULTING DOSE & EFFECTS.		M.C.D. before	M.C.D. after.
B. & W. Russ. ♂ 1600g.	20 days after trace precipi- tin.	5 cc Horse Serum. intra- venous no symptoms.	.2 cc.	(.2 cc. bled 10 minutes after injection.	
White ♂ 1800g.	fresh animal.	Horse Serum 5 cc. intra- venous: no symptoms.	-	-	
"	10 days after strong precipi- tin.	Horse Serum 2 cc intra- venous: extreme pros- tration.	.05 cc.	.15 cc. bled 2 hrs after injec- tion.	
"	6 hours after no precipitin.	Horse Serum 4 cc. intra- venous: no symptoms.	.1 cc.	.1 cc.	
"	20 days after strong preci- pitin.	Horse Serum 5 cc. intravenous; severe symptoms.	.15 cc.	.4 cc (nearly complete) bled 50 minutes after injection.	

together It may be objected that this fall in complement strength has not necessarily occurred 'in vivo' but may arise in the coagulating blood 'in vitro' as a result of the combination there of antigen and precipitin. Michaelis and Oppenheimer (58) are responsible for the statement that precipitation 'in vivo' does not occur. The reasons put forward are (1) that intravenous injection of antigen in animals whose serum is rich in precipitin is not followed by the appearance of intravascular clots: (2) that no disturbance is perceptible in the animals thus injected except a polymorphonuclear leucocytosis, occurring some time after. But the processes of precipitation and blood coagulation have nothing in common and the precipitum resulting from the combination of antigen and precipitin differs so little in consistence from the serum in which it occurs that mechanical effects cannot take place. (The granules which make up the precipitin are extremely minute as can be seen by adding to a drop of whipped blood taken from a precipitin animal a little of the appropriate antigen and watching under the microscope: the granular appearance that results is just capable of resolution into particles with the highest magnification: later those run together/

together to form masses readily broken up by agitation). Secondly the experiments already recorded in this paper controvert sufficiently the statement regarding the absence of symptoms.

That there has been interaction between the two substances is shown by the behaviour of the precipitin, its absence from the serum immediately after the antigen injection. That it takes place not 'in vitro' but before the blood is drawn, is shown by the fact that the complement strength rapidly rises to normal as the animal recovers: the precipitin cannot now, therefore, be acting in the coagulating blood but must have been thrown out from the plasma before the bleeding; (antigen can still be demonstrated in the serum in abundance for an 'in vitro' reaction if such occurred.)

GENERAL STATEMENT.

The general statement can thus be made that intravenous injection of foreign serum in an animal whose blood-serum contains the specific precipitin is followed (1) by symptoms whose severity is proportional to the strength of precipitin and (2) by a depression of the complement property of the/ on/

the animal's fresh serum the duration of which coincides with the persistence of the symptoms.

CERTAIN IRREGULARITIES.

Animals whose respective sera differ only by slight degrees, - all possessing a moderate amount of precipitin, - when injected intravenously with small doses of the appropriate foreign serum display symptoms whose differing severity judged 'clinically' does not correspond with the differences of precipitin. Some other factor must come into play. In the first place the precipitin estimation is conducted with diluted antigen; This is not strictly comparable with the process occurring on injection in the circulating blood where undiluted antigen is rapidly mixed with the precipitable protein. 'In vitro' such a mixture has different effects according as the two constituents are allowed to mix spontaneously or are immediately stirred: the relative proportions of the two also are of importance. For example if equal quantities of undiluted antigen and precipitin serum are promptly intimately mixed, no precipitation appears though usually there is a slight turbidity: curiously too there is only partial fixation/

fixation of complement although there may be a hundred times more antigen present than is necessary in the diluted condition to cause copious precipitate and complete complement fixation.) If to the precipitin serum one-tenth of its volume of undiluted antigen is added (i.e., so that the precipitin is in marked excess) and promptly mixed considerable precipitation appears and the complementing power of the (fresh) precipitin serum is destroyed.

It was thought that the sensitiveness of the animals' complement under these conditions i.e., the degree to which it was affected 'in vitro' by undiluted antigen, might coincide with the severity of the symptoms; the precipitin serum was taken fresh and to it undiluted antigen was added in the proportions corresponding to the mixtures that would result on intravenous injection of the doses employed e.g., a dose of 3 cc. foreign serum in an animal of 2,000g (with therefore a blood volume 100cc. and serum say 50 cc.) would correspond roughly to a mixture, 1 of undiluted antigen to 20 of precipitin. The amount of lytic power (for sensitised red corpuscles) which remained after this treatment was then determined. There were marked variations with the different sera in the proportion of/

of antigen giving maximum complement destruction but the categorical arrangement deduced from experiments with a given dose of antigen practically coincided with that based on precipitin strength. (The slight irregularities observed do not impair the generality of the statement above that strong precipitin involves severe symptoms, weak precipitin slight and transient disturbance; an explanation of their possible origin will be given in the discussion of the etiology of the anaphylactic syndrome).

385 IS THERE A CONNECTION BETWEEN THE DIMINUTION
 killed to OF COMPLEMENT AND THE SYMPTOMS?

is practically imp —————
 advanced? The diminished complement strength depends directly, it would seem, on the interaction of antigen and precipitin in the circulating blood. Is it possible to link this up further with the production of the symptoms? The fact that the period of weak complement synchronises with that of illness certainly raises the question of a causal connection. It has already been mentioned (p. 26.) that complement-weakening 'in vivo' by means of an antigen so different as washed red corpuscles was associated with somewhat similar symptoms. Has the change in the physical or chemical condition of the serum, reflected/

reflected in the loss of its active lytic power for formed cells, had as a consequence the train of symptoms described? The data furnished by the anaphylactic animals are frankly insufficient for this assertion. Initial difficulties in obtaining these data are (1) the difficulty of obtaining serum at precisely the same period of illness in different animals or in the same animal on different dates (since it is necessary to allow the illness to complete its course before an estimate of its severity can be arrived at (i.e., the animal can not be killed to get blood) while bleeding from the ear is practically impossible before recovery is fairly advanced). (2) The marked variation to begin with in the complement-strength in different individuals. As a consequence, though the serum of the same rabbit before and after illness can be compared with considerable accuracy, it is impossible or illegitimate to compare the one rabbit with the other. The figures at my disposal indicate, it is true, that, roughly, the extent of complement destruction is proportional to the severity of the symptoms. In those animals which died, for example, the serum is practically inactivated, while in those which had slight symptoms there may be little difference in the lytic/

lytic power before and after.

On the other hand among those rabbits which have undergone prolonged treatment (some months) without producing strong precipitin I have seen the serum almost inactivated although the symptoms had been only moderately severe. An animal thus may continue to functionate with a serum differing absolutely from the normal serum in one of its most important properties. Interesting as this observation is in itself it certainly bears strongly against the theory that the complement property of the blood is the mirror of vitality, its changes reflecting higher and lower planes of vigour in the animal's economy and its disappearance (as e.g. in fresh blood in vitro) signifying the change in the blood from a living to a dead tissue. Were this so, these animals with a serum thus deprived of vitality should have died. On the other hand again is the fact that on using a considerable variety of agents, complement destroying 'in vitro'; for intravenous injection symptoms appear where these succeed in causing a fall 'in vivo,' none where these fail, as happens, though injected in quantity sufficient 'in vitro' to use up the complement of all the animal's circulating blood. The conditions are obviously very complex; the details of some of these last/

last experiments and some tentative conclusions will be given in the discussion of the pathology of the anaphylactic death.

EFFECTS ON THE BACTERIOLYTIC AND OPSONIC PROPERTIES OF THE SERUM.

Meanwhile it is important to observe that the complement thus affected is not merely the property brought to demonstration by so artificial a procedure as the addition of ox blood corpuscles sensitised by haemolytic immune serum but that the serum during the anaphylactic illness has lost its normal lytic power for bacteria and is presumably open to their invasion. Not only so but the second defensive mechanism the opsonic or bacteriotropic property which throws the invading bacteria within the range of the digesting phagocytes is also put out of action

BACTERIOLYTIC/

BACTERIOLYTIC EXPERIMENTS.

The test organism chosen in the determination of the bacteriolytic strength of the serum was the *Vibrio Nasik*: I am indebted to Dr. Ritchie for the culture. This micro-organism was isolated during a cholera epidemic at Nasik near Bombay: it resembles closely the *Vibrio Cholerae* of Koch but is distinguished by producing in broth a lysis for red blood corpuscles. (The reason of the choice was the intention of employing this haemotoxin as an antigen. In this way the behaviour of the auto-haemotoxin in anaphylactic rabbits might be studied in simple test tube experiments, by 'in vitro' neutralisation of the lysis instead of the costly animal experiment. The small quantities both of toxin and antitoxin produced discouraged one from pursuing this plan.

The method employed was slightly modified from that of Wright (101). An emulsion of the vibrio from an eighteen hour culture at 37°C is prepared with sterile saline (.85 p.c.), and centrifuged to remove clumps: it must be of such a strength that each cubic millimetre contains from 100,000 to 1,000,000 living vibrios: (it is easy with a little experience/

experience to judge of the opacity corresponding to these limits).

By means of sterile graduated pipettes successive decimal dilutions are made of this emulsion with sterile saline. Five of these are prepared from 1-100 to 1-1,000,000 and of each 25 cubic m.m., are taken in a sterile graduated pipette and mixed thoroughly with the same volume of the fresh serum: the mixture is then sealed in the pipette and incubated at 37°C. After the required period - 18-20 hours was found to give maximum bacteriolysis with rabbit-serum but a four-hour period was always used in addition - each mixture is blown out on slope agar and the sterility point determined; the dilution sterilised by the serum gives the number of living vibrios destroyed.

This method is particularly adapted for comparative tests on the bactericidal power of fresh serum - (it is much less useful where the bacteriolytic serum requires to be complemented) - and with the serum of rabbits before and after the anaphylactic illness, striking contrasts are obtained. The normal serum sterilises a dilution containing - it varies in different rabbits - from 20-2,000 vibrios as calculated from plate controls; the serum after injection is invariably overgrown with the vibrio; even the mixture which could not have contained originally more than some units of vibrios,

vibrios, showing an abundant growth.

If the four-hour period of incubation is taken, the differences are already perceptible; with the serum 'before injection' the vibrios are distinctly fewer while with the serum 'after injection' not even temporary inhibition seems to have occurred; in the higher dilutions multiplication is already obvious. In a word there is complete abolition of the bactericidal power.

The full protocol of a typical experiment in which the precipitin the haemolytic complement, the bacteriolytic power and the opsonic power were all estimated will be given after discussion of these last.

THE OPSONINS.

The mixtures of serum, bacteria and washed leucocytes were made with the usual precautions: both rabbit and human leucocytes were used in the first experiments, later human leucocytes alone: these latter are larger, less coarsely granular, and do not clump like those of the rabbit while the results arrange the different sera in precisely the same order, (the absolute values however, being different.) The bacteria/

bacteria used were the *Staphylococcus Aureus* - an old laboratory culture - and the *Tubercle Bacillus* - dead bacilli as supplied for clinical opsonic work. Fifty leucocytes were always counted taken from parts of the film as nearly as possible corresponding in the two smears. With both organisms the results were remarkable, a gross difference appearing between the mixture containing the serum 'before injection' and that 'after injection'. Independent of counting it was easy to see that in the former regular phagocytosis was present while in the latter phagocytosis either seemed totally absent or was confined to one or two leucocytes in a whole film.

I have repeated the experiment more than twenty times i.e., determined the phagocytic count before and after twenty different cases of anaphylactic illness, (the majority of which were not specially severe), and always with a similar result: pooling the leucocyte counts I find the mean number of bacteria per leucocyte with serum 'before injection' is 6.4, the mean with the sera 'after injection' 1.9; extremes were 10.2 'before' as compared with 0.5 'after. while in one case so small a difference as 7.0 'before' and 5.7 'after'. Even this last and all the others were such differences as made the observations/

observations independent of small errors of technique. There is, therefore, a remarkable depression of the opsonic index to be included in the anaphylactic syndrome.

THE PROTOCOL OF AN EXPERIMENT.

- A. Symptoms &c: Rabbit Black ♂ (white blaze forehead) [Had received several injections of Horse serum the last 13 days before]
- at 11 am. bled 4 cc. from ear vein with aseptic precautions.
- at 12.3 p.m. received 2 cc. Old Horse Serum intravenous.
- 12.5 p.m. panting respiration: continues rather languid
- 12.20 p.m. practically recovered bled easily 4 cc. from ear vein as before.

Rabbit Black & White Russian ♂ [had received several previous doses of Ox Serum the last 13 days before]

- 11.10 a.m. bled 4 cc. from ear vein with aseptic precaution.
- 12.30 p.m. received intravenous 2 cc inactivated (56°) Ox Serum.
- 12.32 p.m. marked panting.
- 12.38 p.m. recovery commencing - ears become congested.
- 12.42 p.m. bled 4 cc from ear vein as before..

Four samples of blood Blaze 'before' and 'after', Russ. 'before' and 'after', kept at room temp. overnight: serum/

B. PRECIPITIN.

serum 'before' separated, spontaneously,
 serum 'after' not: Clots centrifuged to
 separate:

Horse Serum	1-10	1-100	1-500	1-1,000	1-5,000	
Blaze 5 min.) 'before'	marked turbidity.	turbidity	slight turbidity	?	?) moderate,) rather weak,) precipitin.
24 hrs.)	deposit	small de- posit	large floc- cul.	floccul	Nil)
'after' 24 hrs.	No de- posit.	No de- posit.	No deposit	-	-	no precipitin.
<u>Ox Serum</u>						
Russ. 5 min.) 'before'	marked turbidity.	marked turbidity	turbidity	turbidity	Nil) moderate
24 hrs.)	large de- posit	deposit	small de- posit	large floccul	minute floccul) precipitin.
'after' 24 hrs.	No de- posit	no de- posit	no de- posit.	-	-	no precipitin

Moderate precipitin with each: with a larger dose than 2 cc. probably both
 would have been severely ill: symptoms with 2 cc were slight, not decisively
 more in one than the other.

C. COMPLEMENT ESTIMATION.

TUBE	COMPLEMENT	H.I.B. 1 1/4 M.H.D.)	SALINE	WASHED OX CORPUSCLES (1 p.c. suspen- sion).	Put in incubator at 4.30pm. Read at 6.30 p.m.
Blaze	(1) .25 cc. +	.025 cc.	.75 cc. +	1 cc.	complete M.C.D. = .25 cc.
Serum 'before'	(2) .2 " "	" "	.8 " "	" "	+++
	(3) .15 " "	" "	.85 " "	" "	+++
	(4) .1 " "	" "	.9 " "	" "	+ slight lysis.
Blaze	(5) .5 " "	" "	.5 " "	" "	+ slight lysis M.C.D. = above .5cc
Serum 'after'	(6) .3 " "	" "	.7 " "	" "	trace lysis?
	(7) .2 " "	" "	.8 " "	" "	Nil.
	(8) .1 " "	" "	.9 " "	" "	-
Russ.	(9) .25 " "	" "	.75 " "	" "	complete M.C.D. = .25 cc.
Serum 'before'	(10) .2 " "	" "	.8 " "	" "	+++
	(11) .15 " "	" "	.85 " "	" "	+++
	(12) .1 " "	" "	.9 " "	" "	+ slight lysis.
Russ	(13) .5 " "	" "	.5 " "	" "	+ slight lysis M.C.D. =
Serum 'after'	(14) .3 " "	" "	.7 " "	" "	trace
	(15) .2 " "	" "	.8 " "	" "	-
	(16) .1 " "	" "	.9 " "	" "	-

There is thus a definite drop in Complement.

D. BACTERICIDAL POWER.

Emulsion in sterile saline of 20 hour culture of V. Nasik: of this decimal dilutions made from 1-100 - 1 in million: of each of these 25 c.m. mixed with 25 c.m., of serum and incubated at 37°C: cultures made by blowing out the mixtures and spreading on the surface of nutrient agar after 4 hours and 20 hours.

4 HOURS.	20 HOURS	EQUIVALENT NO. BACTERIA
uniform stippled growth.	abundant growth	15,000
isolated colonies (nutrients)	"	1,000
some loss of col. - weak growth.	"	100
two large colonies	sterile	10
W.L.	sterile	1 or 2
uniform stippled growth.	abundant growth.	as above
large colonies.	sterile	
uniform film of growth.	abundant growth.	

D. BACTERICIDAL POWER.

MIXTURE	4 HOURS.	20 HOURS	EQUIVALENT TO BACTERIA
Serum 'Blaze'			
1 = 1-100	uniform stippled growth.	abundant growth	12,000
2 = 1-1,000	isolated colonies (hundreds)	" "	1,200
before injection	some tens of colonies.	weak growth.	120
3 = 1-10,000			
Serum 'Blaze'			
4 = 1-100,000	two large colonies	sterile *	12
Sterile point *	Nil.	sterile	1 or 2 ?
5 = 1-1,000,000			
Serum 'Russ'			
6	uniform stippled growth.	abundant growth.	
7 Dilutions	three colonies.	sterile *	
8 in above	three "	"	as above
before injection *	one colony	"	
9 order			
Sterile point	none	"	
10			
Serum 'Blaze'			
11 Do	uniform film of growth	abundant growth.	
after injection			

MIXTURE	4 HOURS.	20 HOURS	EQUIVALENT TO BACTERIA.
Serum 'Blaze'			
(12) do.	uniform film of growth.	abundant growth	as above.
(13)	" " "	"	
after injection (13)	" " "	"	
No Sterile point (14)	very numerous colonies.	"	as above.
(15)	thousand colonies.	"	
(16)	uniform film of growth.	"	
(17) do.	" " "	abundant growth	as above
Serum 'Russ'	many thousands of colonies.	"	
(18)	many hundreds of colonies	"	
after injection (19)			as above
(20)	some 500 colonies.	"	
no sterile point.			
Control 1 = 25 c.m. Dilu. 1-1,000 plated out at once = 1,200 colonies.			
Control 2 = 25 c.m. Dilu. 1-million mixed with old sterile = abundant growth.			
Horse Serum for 20 hours then cultivated.			

Serum 'Blaze' before injection destroyed some 10-20 bacteria in 20 hours, the same after injection permitted the one or two bacteria introduced to multiply as rapidly as in the best culture fluids.

Serum 'Russ' before injection, initially much more strongly bacteriolytic, destroyed one or two thousand bacteria, the same after injection showed not even a definite preliminary inhibition but acted as an excellent culture medium.

E. OPSONIC ACTION.

Staphylococcus Aureus emulsion from 12 hour culture at 37°C - centrifuged.

Own Leucocytes washed in .85 saline 4 times.

Sera stood overnight at room temperature in contact with clot.

Mixtures incubated 20 minutes at 37°C:

Mixture/

E. OPSONIC ACTION.

54.

Mixture 1.	20	23	22	17	22	18	75
	50933-300,16,4	450,11,2	30347	11,0930	02,12,40	22,30,19,2,2.	
= Serum Blaze before	65085	20,12,12,1	3,2,17,13,14	= 297 = 5.9			
Mixture 2.	9	12	12	18	13	7	6
	60021 35400	42420	47502	11,0200	02122	06200	02220
= Serum Blaze after	00,11,2,7	07232	00005	20524	00020	= 139 = 2.1	
Mixture 3.	=	7.7		Mixture 4.	=	2.5	
= Serum Russ. before				Serum Russ. after			
Tubercle Bacillus emulsion: other factors as above.							
Serum Blaze before = 1.7				Serum Him. Russ. before = 6.6			
" " after = 0.3				" " after = 1.2			

(The counts are not given in detail: they were all arranged in somewhat similar fashion to those given in full except that the mixture with serum 'after' usually showed a larger proportion of cyphers, i.e., polymorphs without bacteria, the size of the average depending on a few polymorphs which contained each a large number of bacteria). In any circumstances so that no definite conclusion can be drawn].

The fall in the opsonic activity though not so marked as in many of the experiments where symptoms were more severe is sufficiently definite: associated as it is with a temporary leucopenia as will be shown shortly, the leucocytic defensive mechanism of the circulation must in consequence be profoundly affected.

the delayed and imperfect fibrin-formation in the
[To prove by direct experiment that during the anaphylactic depression an animal is open to infection entails the preparation of a bacterial strain which, inoculated intravenously would produce or not according to the dose and the resistance of the animal, a fatal septicaemia. This was regarded as too much of a side issue in the present research though one or two experiments were performed. Considerable doses of the *Vibrio Nasik* were injected intravenously into/

into anaphylactic animals and into controls receiving Horse-serum for the first time. In all however the blood was free from bacteria in $\frac{1}{2}$ -1 hour, and no definite delay in the disappearance could be made out in the former as compared with the latter; it is probable that this organism was not capable of causing a blood infection in any circumstances so that no definite conclusion can be drawn].

'setting', i.e., cessation of fluidity on tilting the vessel. DELAYED COAGULATION OF THE BLOOD. 15-20 minutes.

Besides the changes in the condition of the blood already recorded during the anaphylactic illness there are others less pronounced. Of these the delayed and imperfect fibrin-formation in the drawn blood must be noted. This is never the marked phenomenon observed after small doses of snake venom or intravenous injection of organ extracts. The blood always sets to a jelly within at most 15-20 minutes after withdrawal; the condition of blood fluid in vitro for hours is never found. (The blood in the heart and vessels remains fluid however, for prolonged periods and post mortem clot is rarely seen).

Comparative experiments on the coagulation-time/

time are hampered on this account, since the end-point of fibrin-thread formation does not appear. The normal coagulation time of rabbits as estimated by the appearance of this thread (McGowan's capillary tube method [56]) lies between 6 and 8 minutes; during the anaphylactic depression and for some time after recovery no fibrin thread appears; the coagulation, estimated roughly by the appearance of 'setting', i.e., cessation of fluidity on tilting the vessel containing the blood, occurs in 12-20 minutes.

No direct experiments have been made as to the nature of this delay and imperfection in coagulation. It seemed probable that actual diminution in the quantity of fibrinogen might be responsible since in the process of specific precipitation this latter protein might be thrown down to some extent along with the globulin precipitate.

Such behaviour is known to occur during precipitation in mixtures of proteins. The weak friable nature of the clot was in favour of this hypothesis but against it is the fact that on addition of normal serum or of organ extract, a second fibrin formation often occurs in the fluid exuded from this clot. This would indicate that some factor in the/

the ferment production is rather at fault. It does not seem that a decision on this point would be likely to throw light on the pathology of the general condition especially as the phenomenon though regular is not very pronounced. The fact of its existence however, is of some theoretical importance as will be seen later.

THE BEHAVIOUR OF THE LEUCOCYTES.

If the leucocytes are counted immediately before the anaphylactic shock and again during the stage of depression - (as soon as a sufficient drop of blood can be got from the anaemic ear) - the difference is remarkable; a drop of 30 to 60 p.c. frequently occurs [with a first injection of foreign serum in moderate quantity no such change appears]. Some examples of such counts may be given.

(1)./

(1). Himalaya ♀ weight 1700g: last dose Horse Serum 12 days before: moderate precipitin:

Leucocytes at 12 noon = 14,000 per c.m. Received $2\frac{1}{2}$ cc. Horse Serum intravenous at 12.40

Leucocytes at 12.48 p.m. = 6,000 " " symptoms mild but definite.

(2). White ♂ 2000g one previous dose of Horse Serum 13 days before - very strong precipitin.

Before (leucocytes at 4 p.m. = 10,000 per c.m.
injection)

at 3.40 p.m. bled 2 cc.

" 4.10 p.m. = 8,500 "

At 4.52 intravenous injection $2\frac{1}{2}$ cc Horse Serum.

" 4.20 p.m. = 12,000 "

very severe symptoms & death at 5-6 p.m.

" 5.7 p.m. = 3,000 post mortem.

Blood from heart taken for count.

(3). Black ♂ 1820g first dose Horse serum 13 days before: slight precipitin: cf. last animal.

Leucocytes at 12.45 p.m. 18,000 per c.m.

At 1 p.m. bled 2 cc.

" 2.15 p.m. 16,500 per c.m.

at 2.35 $2\frac{1}{2}$ cc. Horse serum intravenous

" 2.43 5,500 per c.m.

symptoms slight, recovery in 10 mins.

= 8 minutes after injection.

This temporary leucopenia appears not merely after intravenous injections in which the foreign material thus suddenly brought into direct association with the blood cells might be responsible but also on injection into the peritoneum though not to the same degree. For example:- Black ♀, received 2 previous injections of ox serum, the last 8 days before;

10 a.m. leucocytes 10,500 per c.m.

10.15 a.m. received 5 cc. inactivated ox serum intraperitoneally symptoms very slight - some panting.

10.50 a.m. leucocytes 4,000 per c.m.

12 noon " 4,600 "

4.30 p.m. " 7,200 "

6 days later again subjected to this treatment.

10 a.m. leucocytes 11,600 per c.m.

2 p.m. intraperitoneal injection of 5 cc. inactivated ox serum.

4 p.m. leucocytes 4,000 per c.m.

7 p.m. " 9,400 "

This phenomenon does not occur with the first dose of foreign serum. It must be noted, however, that the leucopenia does not appear with the same regularity as the diminution of complement. Occasionally - and no rule can be discovered for its occurrence - there is either no fall in the number detected/

detected or an increase well outside the limits of experimental error occurs. Yet in these animals the usual train of symptoms and drop in complement took place. (This fact is strongly against the explanation which might be furnished of the fall of complement that it depends on the leucopenia, a blood clot containing few leucocytes should on the leucocytic theory of the origin of complements express a serum poor in this property but one with the number of leucocytes unimpaired might be expected to furnish complement as strong as before.)

THE DIFFERENTIAL COUNT.

The polymorphonuclear leucocytes are chiefly affected in this leucopenia. A blood film made while the animal is in the stage of acute illness contains practically no polymorphs or a few clumps strongly agglutinated. Considering the extremely sluggish circulation in the ear vein in this condition it might be thought that the actively amoeboid polymorphs had gravitated to the vessel wall so as to produce the apparent disappearance but films made from the heart blood of fatal cases and also from the portal vein are equally poor in polymorphs. The condition/

condition of strong agglutination is very striking, even in the blood diluted for enumeration, which has been vigorously shaken, marked clumping appears among the (few) leucocytes got in the counting field.

The significance of this leucopenia is precisely the same as that of the delayed blood coagulation i.e. it brings the anaphylactic syndrome into relation with the phenomena known to appear with certain organic poisons.

THE ANAPHYLACTIC REACTION TO SUBCUTANEOUS INJECTION.

No experiments have been made on this. The condition had already been studied in rabbits and detailed histological descriptions given both by Arthus and Breton (3) and by Thompson and Marchildon (94). Briefly it consists of intense local oedema with haemorrhagic necrosis of the connective tissue; this results in the formation of large ulcers healing very slowly. I can confirm Arthus and Breton's statement that injection under the skin of the ear on the other hand does not lead to ulceration but to a chronic granulomatous swelling.

THE TIME RELATIONS.

After a first dose of foreign serum, no anaphylactic reaction can be elicited before the eighth

eighth day; by the tenth day the reaction may appear in full vigour. The period of maximum sensitiveness extends from the twelfth to the twentieth day, at later intervals severe symptoms become more and more rare.

After the second and each subsequent dose there succeeds a period of from three to five days when no symptoms are induced by any dose of the foreign serum. The sensitive animal has become 'refractory'. On the fifth day symptoms are usually obtainable and by the sixth as a rule a severe reaction again appears. The period of maximum sensitiveness in these reinjected animals lies from nine to sixteen days from the last dose. (Occasionally it occurs that a small assaulting dose fails to rob the animal of all its precipitin with the result that the 'refractory' period is only one of relative insensitiveness; mild but definite symptoms appear on reinjection under these circumstances. Absolute refractoriness is however the rule). The time relations which exist between the appearance and severity of the symptoms on reinjection and the period elapsed from the last dose agree remarkably with those given for the appearance of precipitin after injection. The factor of time seems quite subordinate to that of the amount of the precipitin. (The conditions/

conditions of anaphylaxis in the guineapig are strikingly different as will be seen later.)

THE PROTEINS PRODUCING ANAPHYLAXIS.

The effects described may be produced by proteins widely different in origin. Among those I employed, were blood serum, (horse, ox, human), milk (cow's), gelatine, the protein of rice, blood corpuscles (washed free from serum), bacteria (in cellular form) and bacterial protein (freed from cells by filtration through a Berkfeld filter).

In the character of the symptoms produced there is no essential point of difference with any of these. With each and all the typical behaviour appears on repeated doses. There are, however, certain effects wherein several of these substances differ from horse-serum and from each other. Horse-serum, as has been said, however fresh seems absolutely devoid of toxicity; neither immediate nor remote effects are observed from large doses. (In man this is not so, symptoms appearing with even a first dose, in the form of urticaria arthritis etc., but the difference here also does not depend, as will be shown, on a primary toxicity of the serum but on a vital/

vital reaction of the human organism). The serum used was, in general, normal horse serum, bled with aseptic precautions and preserved at room temperature in sealed flasks. Experiments were also done with horse serum, antitoxic for diphtheria, which dated some thirteen years back. It could not be said that any difference existed, this serum was certainly as fatal as the more recent. Ox serum on the other hand when fresh, possesses a strong original toxicity. The lethal dose for rabbits of average weight by intravenous injection is about 5cc. The effect observed is superficially like the anaphylactic death, a fatal termination about 10 minutes after injection. But the differences are distinct, the animal after injection usually appears quite unaffected; there is no prostration; the ears are hot and congested with marked distension of the veins instead of pallor. Without warning the animal ceases to breathe, falls on its side with convulsive movements and after one or two gasping cries expires; the appearance is that of an acute asphyxia. Post mortem there is congestion of the abdominal viscera and an intense oedema of the lungs. There is a definite fall in the complement strength of the serum. Blood obtained post mortem, as it clots, shows strong corpuscular agglutination; this only develops, however, after/

after removal from the heart.

Ox serum, inactivated by 1 hour at 55°C, is apparently as non-toxic as horse-serum, although it still possesses its agglutinating powers for rabbits' corpuscles practically unaffected. I am inclined to think that it produces death by anaphylactic shock more readily than does horse-serum, resembling in this human serum. To decide this point, however, a very large number of animals would be necessary owing to the extreme variation in susceptibility among individuals.

Human serum when fresh, is also toxic but to a less degree, the lethal dose lies nearer 10 cc. than 5 cc.; the symptoms are the same as with fresh ox serum.

Milk has no inherent toxic property; large doses may be given without symptoms. It was used as a solution of caseinogen the lactalbumin being removed by heat. In the three animals treated it produced anaphylactic symptoms definite but much less severe than with blood-serum; no death occurred even after prolonged treatment nor did more than very slight precipitin develop.

Gelatine failed to produce symptoms after prolonged trial. The two animals used, however, were poor precipitin producers and also failed to react to horse-serum/

horse-serum so that the question of its capacity to produce anaphylaxis was left unsettled.

Rice-protein was prepared from the millings of rice. These contain a large proportion of the total protein of the rice seed. A watery extract was filtered clear and precipitated by saturation with ammonium sulphate. The precipitate was washed with a saturated solution of the same salt and dialysed against running water till free from ammonium; a proportion of the precipitate remained insoluble. Sodium chloride was added to make the fluid isotonic and it was then filtered through a Berkfeld filter. A considerable number of experiments were conducted with this in connection with another research on the 'rice theory' of Beri-beri and the possibility of the extract producing a neurotoxic action. The rice protein was found to possess a definite deleterious effect on metabolism, the animals into which it was injected lost weight for several weeks after, and two of them died with extreme emaciation but no other definite symptom. On repeated injection specific precipitin was produced and the typical anaphylactic symptom-complex in proportion. No apparent immunity to the emaciating effects of the rice-protein developed in these animals. An extract of milled rice furnished a similar protein in smaller quantity. Animals were fed on rice for two/

two months without developing a trace of precipitin nor could any trace of rice-protein be detected in their serum by means of the specific precipitin. This is in contrast to the effect of feeding with ox serum with which traces of precipitin are fairly easily obtained.

With washed ox corpuscles a large number of observations were made. Certain differences in the anaphylactic syndrome appear. Immediately after the assaulting injection in several of the sensitive animals convulsions or staggering movements occurred; these were then followed by the typical stage of depression which might end by recovery after an illness decidedly more prolonged than the usual serum depression, or by death with the appearances described.

The reaction demands a longer interval from the last dose than with serum. If the dose is repeated under 12 days there may be no effect: the maximum sensitiveness seems about the eighteenth to the twentieth day. Besides this more or less typical anaphylactic reaction there is another form of hypersensitiveness which is more chronic in course. After the period of acute depression has passed the animal remains languid, refuses food and dies in the course of 24-36 hours. Intraperitoneal injection of the corpuscles although it prevents the typical immediate syndrome from acting fatally, does not prevent this/

this delayed death. The animal is found the day after injection dying with a subnormal temperature as in the last stages of an acute infection, but with blood and peritoneum sterile. It is this form of hypersensitiveness which is responsible for frequent disappointments in the preparation of strong haemolytic serum. The danger from it seems to increase as the haemolytic strength rises, and has little relation to the length of the period elapsed from the last dose (on which the appearance of the acute phenomena depends).

Also, the acute death may be obviated by a small dose given some little time previous to the large one as has also been pointed out by Besredka (8), but the late death may still occur in spite of this. As with bacteria one is compelled to assume the liberation of some toxic substance from the lysed corpuscles to account for these results; such delayed effects very rarely appear with foreign blood serum. (I have seen only one case and that not actually fatal. In this, after a total dose of 10 cc. of horse-serum given in separate small quantities intravenous in the course of a day to a rabbit with strong precipitin, the animal became gradually very ill and remained prostrate with shallow breathing, and subnormal temperature for 48 hours; thereafter it/

May 25th. (3 weeks after) it slowly recovered to become apparently normal in the course of three days).

With Bacteria the typical anaphylactic syndrome appears regularly on intravenous injection in the course of immunisation. The symptoms, however, though often severe, have never in my experience produced the typical death; the animal after a period of prostration recovers to fall victim to a slowly increasing depression during the succeeding four hours. It is important here to distinguish between the immediate anaphylaxis and the hypersensitiveness which is evidenced by death some hours after the assaulting dose. They have, I believe, a different cause and certainly the post mortem appearances are markedly divergent.

The notes of an experiment may be submitted:-

May 7th. Smut ♂ Weight 1950g, fresh rabbit, received intravenous.

11.30 a.m. .5 cc. of a thick emulsion of *Vibrio* Nasik (killed by heating to 70°C) - no symptoms $T^{\circ} = 102.5^{\circ}\text{F}$.

2 p.m. animal languid but restless $T^{\circ} = 103.4^{\circ}$.

3 p.m. depressed, languid appearance, $T^{\circ} = 103.2^{\circ}\text{F}$.

4 p.m. brisker looking $T^{\circ} = 102.8^{\circ}\text{F}$.

4.45 p.m. recovered, feeding $T^{\circ} = 102.2^{\circ}\text{F}$; bled 1cc; no haemoglobin in the serum.

May/

May 28th. (3 weeks after) .3 cc V. Nasik emulsion intravenous; behaved very much as last time.

June 18th. (3 weeks later).

9.30 a.m. bled $2\frac{1}{2}$ cc. from ear.

11.15 a.m. received intravenous 1cc V. Nasik emulsion.

11.16 a.m. very restless: panting.

11.17 a.m. prostrate, panting, muscular weakness marked.

11.20 a.m. comatose condition: hardly reacted on disturbance.

11.25 a.m. recovering slightly.

11.30 a.m. sitting up: moving about: still breathing heavily.

12.30 p.m. decidedly languid: lies at full length.

1.30 p.m. looks ill: refuses food: moves about feebly.

3 p.m. recovering: more lively.

5 p.m. practically recovered.

The two stages of illness were quite definite and separated by a period of about half-an-hour during which the animal appeared normal. (At a later experiment the animal died six hours after the assaulting dose with markedly subnormal temperature. The Histology of the organs will be described later.

Bacterial protein free from cell elements was prepared from a thick emulsion of the *Bibrio* Nasik in saline. This was vigorously shaken at frequent/

frequent intervals for several hours during which it was kept in a water bath at 50°C. The extract thus prepared was filtered sterile through a Berkfeld filter; it contained a definite quantity of coagulable protein and produced with the immunised rabbit's serum the typical precipitation phenomenon. (This is to be distinguished from the precipitation of bacterial extracts by immune serum where these extracts have not been filtered. In these a large part of the precipitum consists of bacterial protein while with the filtered extract the precipitum, as with horse-serum, reflects almost entirely the amount of precipitable protein in the antiserum). With such an extract, definite though slight immediate symptoms appeared in the immune animals on intravenous injection and the usual fall in the strength of the complement, the bacteriolytic and the opsonic properties.

THE CONTROL WITH HOMOLOGOUS SERUM.

An important control, especially for the experiments with foreign blood-serum, is that with normal rabbit serum. Repeated administration of this (homologous) protein has no definite effect.

Dec. 31st. Black and white ⁰ + 1450g received 10cc. normal rabbit serum intravenous - no symptoms.

Jan. 14th. bled 5cc. at 1.30 p.m.; received intravenous 5 cc. normal rabbit serum (same serum kept frozen since Dec. 31st) - no symptoms - bled again 5 minutes after injection; complementary dose same as before injection.

Jan./

Jan. 28th. bled 2 cc. at 12.30 p.m.; at 12.40 p.m. received intravenous 5 cc. normal rabbit serum (fresh from different animal) - no symptoms.

Feb. 7th. bled 2cc. at 11 a.m.; at 11.30 a.m. received intravenous 5cc. normal rabbit serum (same as last; kept frozen) - no symptoms, bled 2 cc. at 11.30 a.m. - clot did not separate serum spontaneously. Minimum complement dose same before as after injection; at 4 p.m. received 4 cc. ox serum intraperitoneal.

Feb. 17th. bled 2 cc. at 12 noon - serum contained slight precipitin - at 12.30 p.m. received 2 cc. ox serum intravenous - definite symptoms - prostration lasting 8 minutes.

This animal thus, though capable of producing precipitin and of reacting with the usual symptoms to foreign blood serum showed no disturbance on repeated injection of normal rabbit serum.

(With homologous organ extracts on the other hand I have an isolated observation - not worked out in detail - where definite, fairly severe, symptoms appeared on repeating intraperitoneal doses).

Nature of the process could be attempted. My experiments on this

PASSIVE TRANSFERENCE.

guinea-pig are confined to some 20 guinea-pigs, but with

With moderate doses of a strong precipitin serum and the specific antigen it is easy to reproduce severe degrees of prostration in fresh rabbits.

I have not observed a fatal result. (Such has been recorded recently by Briot (17) but there can be no doubt/

and Hagerau and Anderson (18) in the same year published observations on the "Theobald Smith

phenomenon".

doubt that the symptoms by the passive method run a decidedly milder course). The notes of an experiment with the necessary control will be found in the section devoted to Respiratory Exchange (p.106.). A blood-pressure tracing under the same conditions will be found in Volume II.

SERUM-ANAPHYLAXIS IN THE GUINEAPIG.

The conditions under which serum-anaphylaxis - the Theobald Smith phenomenon - appears in the guineapig had already been thoroughly studied by various observers. These conditions differ remarkably from those in the rabbit as do the symptoms to a considerable extent. It became necessary to examine these differences before any generalisation as to the nature of the process could be attempted. My experiments on the comparative behaviour of rabbit and guineapig are confined to some 25 guineapigs, but with these I have been able to confirm most of the important points mentioned in the literature.

The first communications on the effects of repeated serum administration in the guineapig appeared practically simultaneously R. Otto (65) in 1906 and Rosenau and Anderson (83) in the same year published observations on the "Theobald Smith phenomenon"./

phenomenon". This phenomenon had been observed for some years by Theobald Smith (see Lewis (54)) in the course of estimating the antitoxic value of anti-diphtheria serum; sudden death or severe illness appears on injecting horse-serum subcutaneously into guineapigs which have received some weeks previously an approximately neutral mixture of diphtheria toxin and antitoxin (i.e. horse-serum). These authors found that the Toxin element of the preliminary treatment played either no part or only a minor one in the production of the hypersensitive condition: normal horse-serum alone was certainly capable of producing it in full degree. The two points demanded are (1) a minute dose of the serum preferably not over $\frac{1}{100}$ cc. (a dose as small as one-millionth of a cc. has produced the condition), (2) an interval of not less than 10 days and preferably three weeks before the assaulting dose. This latter if administered subcutaneously or intraperitoneally must be large, 5-10 cc., but if intravenous, intracardiac, or intracerebral (Besredka (8)) need not exceed 0.25 cc. - all these doses being harmless in the normal animal. In animals to which large doses are given to begin with, and also in sensitive animals which recover from the illness produced by the large assaulting/

assaulting dose, a condition of 'antianaphylaxis' Besredka and Steinhardt (9) or 'refractoriness' (Gay and Southard (37)), is set up in which for many weeks the animals behave to assaults with horse-serum as normal animals but eventually become again hypersensitive.

SYMPTOMS IN THE GUINEAPIG.

The symptoms are very characteristic.

Notes from two of my own experiments are submitted.

FATAL ANAPHYLAXIS.

Guineapig series (3)

Black (brown saddle) $\frac{0}{+}$ weight 470g had received $\frac{1}{50}$ cc. horse-serum intraperitoneal 40 days before - developed no precipitin.

3 p.m. bled $1\frac{1}{2}$ cc. from ear (using the same technique as for rabbits) (see illustration p.).

3.7 p.m. received 1 cc. warm horse serum intracardiac (readily performed with an ordinary hypodermic needle; the animal must be held perfectly still; the needle is entered at the left edge of the sternum two spaces from its lower end and directed somewhat towards the middle line; it seems to pierce as a rule the right auricle); immediately after injection several spasmodic coughs then severe expiratory efforts.

3.8 p.m. became suddenly limp, fell over on its side with lax swollen abdomen.

3.9 p.m. breathing ceased; strenuous convulsive extensions of the limbs and back muscles, throwing the animal about, occurred thrice in/

in quick succession.

3.10 p.m. corneal reflex gone; heart still beating feebly.

P.M. Heart - right chambers engorged - trace of blood in the pericardium.

Lungs - large, pale, (oedematous?)

Liver - intense engorgement; intestines bright red; stomach intense congestion of cardiac end - petechial haemorrhages sub-mucous.

NON-FATAL ANAPHYLAXIS.

Guineapig series (4)

+
White O 550g - kept without food for 36 hours - (had received 22 days before 100 cc. Horse-serum intraperitoneal)

11.50 a.m. Rectal temperature = 101.2°F.

11.58 a.m. received 5 cc. horse-serum intraperitoneal (warm) - heaving respiration began almost at once.

12.1 p.m. restless; sneezed several times and scratched itself vigorously about the head and body; coat began to 'stare' about the neck.

12.5 p.m. continues very restless with deep heaving respirations; the abdomen is slightly swollen; the animal seems to feel pain when handled.

12.10 p.m. marked shivering movements with 'staring' coat - still very restless; appears to suffer from general itching.

12.30 p.m. continues restless with scratching movements, but obviously feeble; lifted it feels quite limp and tends to lie on its side when at rest.

12.45/

12.45 p.m. lying prostrate; breathing now rapid and feeble Rectal T° = 96.0°F .

1.15 p.m. extremely feeble, lies at full length breathing feebly.

2 p.m. Rectal T° = 89°F . Put in incubator at 30°F .

4.30 p.m. Rectal T° = 95°F . Now decidedly improving

Kept overnight in incubator T° at 7.30 p.m. = 97.4°F .

Next day quite lively T° 101°F ; feeds readily. (Died unexpectedly 2 days later, with general congestion of lungs and abdominal viscera.)

From these descriptions it will be seen that the symptoms in the guineapig are by no means similar at least superficially to those in the rabbit. In the guineapig evidences of asthma and urticaria predominate, in the rabbit a condition more resembling surgical shock. They have in common the cessation of respiration before the heart and the terminal convulsions, the latter, however, much more marked in the guineapig.

THE BLOOD-CHANGES.

A. THE COMPLEMENT.

As might be expected from the conditions of production, so different from those necessary in the rabbit, the blood changes in the anaphylactic guineapig/

guineapig bear no similarity. There is no precipitin to be detected in its serum and after illness so far from having a weaker complement the serum is actually more strongly lytic than before. For example in the fatal case just described the complement estimation before and after death resulted as follows:-

COMPLEMENT.	H.J.B. (dil.1-10)	SALINE	OX COR- PUSCLES. 1%	45 minutes at 37°C.
				Suspension
Serum (1 .05cc. . . 010cc.		.96cc.	1cc.)	complete
Be- (2 .04 " " "		.97 "	")	"
fore (3 .03 " " "		.98 "	")	"
(4 .02 " " "		.99 "	")	partial
Serum (5 .05cc. " "		.96 "	")	complete.
(6 .04 " " "		.97 "	")	"
after (7 .03 " " "		.98 "	")	"
(8 .02 " " "		.99 "	")	"

M.C.D. before = .03 cc.; after = .02cc.

(This is all the more remarkable as the guineapig's complement is readily destroyed 'in vitro' by horse-serum.)

EFFECT OF HORSE-SERUM 'IN VITRO'.

COMPLEMENT.	HORSE-SERUM.	SALINE	H.I.B. 1-10	OX COR- PUSCLES. 1%	1 hour at 37°C.
(Serum 'before')	(undiluted)				
1. .03cc.	.0025cc.	.47cc.	.01cc.	1cc.)	no lysis.
2. .03 "	.005 "	.46 "	"	")	" "
3. .05 "	.005 "	.44 "	"	")	slight lysis
4. .15 "	.005 "	.35 "	"	")	nearly complete
5. .03 "	-	.47 "	"	")	complete

.005cc./

0.005cc. horse-serum thus seriously affects 0.05cc of the guineapig's complement.

(Calculated that a large guineapig has 40cc blood = 20cc serum, then a dose of 2cc horse-serum ought on the 'in vitro' standard to have seriously affected the complement 'in vivo'. Instead of this the serum three minutes after injection is actually stronger than before. The complexity of the complement conditions 'in vivo' is again evident.)

The statements on this question in the literature are contradictory. On the one hand both Otto (66) & Gay and Southard (38) in the course of their search for an anaphylactic anti-body (which should declare itself by combining with horse-serum and thereby as with the known antibodies, fix complement) could find no evidence of altered behaviour in anaphylactic as compared with normal guineapig serum, nor that the serum in the animal undergoing the anaphylactic symptoms was in any way different. The question in view in their experiments being 'complement destruction is it present or not?', it was easy for them to overlook the unexpected phenomenon of a definite if slight increase in the haemolytic vigour of the serum of the pathological animal. On the other hand Nicolle and Abt (63) found definite complement fixation with the appropriate antigen in the serum of sensitive animals as compared with normal animals. The factors in this reaction are so complex/

complex that it is necessary to suspend judgment on the point. There can be no question as to the behaviour I have described in the complement before and after symptoms.

B. THE COAGULATION. RATE.

Unlike the rabbit there is in the blood of the guineapig during or after the anaphylactic syndrome no definite change in the coagulation.

C. THE LEUCOCYTES.

On the other hand there is a very definite leucopenia in at least a certain proportion of the animals.

Guineapig series (4).

Brown and white ⁰ weight 560g (sensitised $\frac{1}{100}$ cc. horse-serum 38 days before) fasting 24 hours.

11 a.m. leucocytes = 7,000 per c.m.

11.30 a.m. " = 15,000 " "

1 p.m. " = 15,800 " "

1.3 p.m. intraperitoneal injection of 5 cc. horse-serum warm, symptoms followed, slight only, chiefly restlessness and heavy breathing.

1.23 p.m. leucocytes = 8,800 per c.m.

2.35 p.m. animal now quite recovered.

" " leucocytes = 30,800 per c.m.

A definite drop with a strong rebound is apparent. This fact has not been recorded as far as I can find in the literature to date. It may have some importance from the point of view of the general consideration of the condition.

THE PROTEINS EMPLOYED.

The typical syndrome has been produced in the guineapig by different observers with a large variety of proteins. In particular Rosenau and Anderson (84) made systematic experiments with proteins of different origin and produced anaphylaxis more or less severe, with the sera of horse, ox, sheep, pig, dog and cat, with haemoglobin, with egg-albumin, milk, with the protein of peas, and with bacteria including coli, tubercle, anthrax, etc. More recently the anaphylaxis to bacteria has been specially studied by Kraus and Stenitzer (50) , Kraus and Doerr, (49) Axamit (5) , Yamanouchi (102) and Delancé (22) , while the tuberculin reaction, undoubtedly a case of hypersusceptibility, has been much studied on lines more or less independent of its position as an instance of bacterial anaphylaxis.

The conditions for anaphylaxis to bacteria differ/

differ considerably from those for the Theobald-Smith phenomenon; they resemble much more nearly those in the rabbit already discussed. Considerable primary doses of the cultures are necessary; the symptoms, on reinjection after a latent period of 10-20 days, resemble closely those in the rabbit, as do the conditions for passive transference.

PASSIVE ANAPHYLAXIS IN THE GUINEAPIG.

Passive production of the Theobald-Smith phenomenon, i.e. transference of the sensitive condition to a fresh animal by injecting the serum of a sensitive one, is not a simple matter as in the rabbit. A latent period is necessary of at least 24 hours after receipt of the sensitive serum before the passive animal reacts to the dose of horse-serum, Otto (66) and Friedemann (33); nor is it possible, as in the rabbit to alter the order of injection, to follow the horse-serum with sensitive serum and still produce the symptoms. On the other hand the hypersensitiveness once set up passively is apparently as permanent as the primary horse-serum effect. The serum of a guineapig itself 'refractory' as the result of a previous assaulting dose strange to say also sensitises passively. Hereditary transmission of/

of the sensitive condition occurs from the mother to the young and this independent of the milk; this is not permanent and is not transmissible to the second generation. It is to account for these peculiar relations that most of the theories of serum-anaphylaxis have been constructed; the important deduction however, is that anaphylaxis must depend on some body, probably of the nature of an anti-body, present in the sensitive animal's serum.

Passive anaphylaxis of an extreme degree may be readily produced as I shall show, in guineapigs with a strong precipitin rabbit serum. A kymograph tracing illustrating this remarkable fact is submitted (Vol. II p.39). In this case no latent period is required nor does it appear to matter whether antigen or sensitive serum be given first. The effects produced are astonishingly similar to the Theobald-Smith syndrome - that is to say, the sensitive rabbit's serum does not always transmit the symptoms of rabbit anaphylaxis but may produce those peculiar to the guineapig. Weill-Halle and Lemaire (97.) alone appear to have tried this experiment, they, however, did not observe suitable conditions for its demonstration; employing subcutaneous doses of the precipitin serum they found guineapigs died in 11 days (symptoms undescribed).

EFFECTS/

EFFECTS OF REPEATED SUBCUTANEOUS INJECTIONS OF FOREIGN SERUM:--

PRECIPITIN FORMATION.

Nicolle (62) and Lewis (54) both record slight degrees of the Arthus phenomenon (i.e. the acute oedema on subcutaneous injection, cf. p.) in guineapigs as the result of repeated administrations of horse-serum. Guineapigs thus treated produce a certain amount of specific precipitin but never more than would be characterised in rabbit serum as very weak. Such guineapigs show no disturbance on injection even with large doses of the antigen. (They tolerate well as a rule, prolonged repetition of horse-serum injections, but I have had several examples of a rapid cachexia under these conditions, and death after some four or five daily doses of 1cc. intraperitoneal).

IN THE RABBIT.

In rabbits dead with the acute symptoms described the histological appearances are very uniform. In all the organs of the splanchnic area THE/ is marked capillary engorgement. In the small intestine/

THE HISTOLOGICAL APPEARANCES.

As might be expected from the rapidity with which the symptoms are produced and recovered from actual anatomical alterations in the cells of the various organs are slight while the chemical changes which may be presumed to occur are not of the sort demonstrable by the ordinary histological methods. The chief changes appear in the vascular tissue.

(Gay and Southard (37) however describe in the anaphylactic guineapig widespread cellular changes. Using Marchi's method they found fatty degenerations - black droplets with the osmic acid in the cells of practically all the organs. The heart muscle showed this particularly well; numerous foci could be seen in which intense fatty change of the muscle cells was obvious. In the other organs the cells most generally affected were not the secreting cells but the endothelial cells. The haemorrhages are due, they say, to rupture of these degenerated capillaries

I have not repeated Gay and Southard's work but have studied the changes in the tissues by the routine methods of fixing and staining. All the material described was removed immediately after death, fixed in warm Zenker's Fluid, embedded in paraffin, and the sections stained with haematoxylin and Eosin).

IN THE RABBIT.

In rabbits dead with the acute symptoms described the histological appearances are very uniform. In all the organs of the splanchnic area there is marked capillary engorgement. In the Small Intestine/

Intestine in particular the free tips of the villi and the folds of mucous membrane are occupied by relatively enormous sacs filled with blood. These sacs are lined by endothelium without special supporting connective tissue. No alteration is perceptible in the cells of this endothelium save the mechanical effect of distension the protoplasm of the cells is stretched to the thinnest rim round the blood-filled space and even the nucleus appears thinned out.

Towards the base of the villi the capillaries may be only slightly more prominent than normal; in the submucous and intermuscular connective tissue however there are venules in a state of extreme distension. Small haemorrhages occur in both situations. the venules in particular often show rupture and interstitial haemorrhage. There is frequently much catarrh of the columnar epithelium but this appears also in normal rabbits to a surprising extent.

In the Omentum and Mesentery the microscope brings out strikingly the acute engorgement already evidenced by the pinkness of these membranes. The venules in particular are large and full of blood while there is a marked excess of blood-containing capillaries. In some cases at each junction of the connective/

connective tissue net work framing the fat cells a minute capillary of the bore of one red corpuscle, can be made out, in marked contrast to the normal tissue where the minute capillary spaces are very difficult to find. The Stomach is less regularly and severely affected: in some cases however the prominence of the interglandular capillaries and venules is very marked: there are relatively wide blood spaces running up between the crypts lined by a slender endothelial layer.

The Pancreas is very markedly engorged with very large dilated venules between the lobules and acini.

The Spleen is in a condition of typical acute congestion: small subcapsular haemorrhages are also found.

In the rabbits liver actual changes in the cells are difficult to be sure of: there are great variations in the staining reactions apparently at different periods after feeding. The engorgement of both portal and sublobular branches is very definite while the intralobular capillaries run as distinct blood filled channels between the cells. In the neighbourhood of the portal spaces peculiar small areas of oedema are occasionally found: in these the cells are separated by the fine granulation of

of a serous exudate.

In the Kidney there is not the same degree of general congestion. The capillaries of the Malpighian tufts are more prominent and full of blood than in the normal animal.

In the Lungs the changes are marked and peculiar. There is an irregular capillary congestion with small haemorrhages into the air alveoli. In great parts of the lung however there is at first sight no congestion, the striking feature is the marked thickening of the alveolar septa. This thickening renders the septa certainly double the normal thickness and gives the lung an appearance of partial collapse on low power view. On minute examination it is seen to be due to an apparent swelling of the cells of the lining endothelium: the nucleus is little altered in size or shape the protoplasm on the other hand is of a peculiar hazy finely granular appearance. The outlines of the capillaries are not sharp: the contained blood corpuscles seem attached and partly fused with the endothelial lining. The alveoli frequently contain a small quantity of serous exudate.

The bronchi are full of mucus as a rule: not infrequently they contain some blood as well.

The arteries appear contracted the veins abnormally/

abnormally full.

S U M M A R Y.

The histological appearances in the anaphlactic rabbit may be summarised as (a) capillary engorgement of the abdominal organs, (b) a peculiar condition of cedema and congestion of the lung.

IN THE GUINEAPIG.

In the guineapig the lung condition takes the prominent place. A condition of typical emphysema is presented; the alveolar septa are of extreme thinness and enclose large air spaces: the capillary channels are imperceptible. Small foci of collapse also occur however in which acute capillary congestion is evident. The breaking up of bronchioles into infundibula can no longer be traced: the terminal bronchioles appear as little masses of densely packed cells scattered among the loose network of thin alveolar walls.

The bronchi are in a state of extreme contraction: even in the largest branches the mucous membrane is thrown into folds closely pressed together and absolutely obliterating the lumen.

The/

The large arteries are much distended, the veins less so.

In the abdomen the Stomach shows the most pronounced changes. A marked congestion of the mucous membrane is present; the venules and capillaries between the glands (especially in the cardiac end) are acutely engorged and ruptures appear with effusion of blood escaping towards the free surface. Intense engorgement of the veins of the submucous connective tissue is also a striking feature. The omentum and mesentery show a varying degree of capillary congestion, sometimes very marked.

The Intestine as a rule displays only a slight degree of distension among the capillaries of the venules: occasionally there is no definite congestion. The Spleen shows marked subcapsular congestion. (A peculiar phagocytic activity for the red cells has been noted in three cases).

There is little definite change in either Liver or Kidney: there is some congestion in both and an appearance in the cells like cloudy swelling.

S U M M A R Y.

Bronchial spasm and its consequences in the lung, congestion and petechial haemorrhages in the abdominal organs, possibly directly due to the acute asphyxia, sum up the histological changes.

THE MECHANISM OF THE SYMPTOMS.

A. IN THE RABBIT.

(Experimental data in this direction existed only for the dog. In March 1909 Biedl and Kraus (12) described the physiological action of large doses of foreign serum intravenous in dogs which had received some time before a sensitising dose of the same serum. The symptoms bear some resemblance to those in the rabbit but are never so severe and very easily cause death. The dog displays sudden muscular weakness with vomiting and prostration; after a short period of restlessness it rapidly sinks into a deep coma, breathing easily and regularly, however, (never any dyspnoea) and reacting to disturbance. The condition may endure some hours during which vomiting and evacuation of the bladder and bowels occur from time to time; thereafter the animals recover perfectly and are 'refractory' for some days after. Kymographic tracings show a prolonged fall of blood pressure which Biedl and Kraus regard as the central point of the symptom complex. To it they ascribe the coma and the vomiting and not to any toxic action on the nervous system. Its mechanism they perceive in a peripheral vasodilatation of such a character that splanchnic and vasomotor centre stimulation have lost their influence and even adrenalin fails to cause a rise of pressure. The plain muscle of the peripheral arterioles, it is held, have been paralysed and no longer produce their pressure-maintaining action on the arterial blood. Barium chloride alone is capable of overcoming this action and produces accordingly rise of blood-pressure and the complete disappearance of the symptoms).

In the rabbit the mechanism is certainly different though the central fact in it, the fall of blood-pressure, is the same and is almost certainly the chief cause of the comatose condition.

A/

A FALL OF BLOOD PRESSURE

The Kymographic tracing (see Volume II. p. 30) shows that synchronous with the appearance of the prostration in the unanaesthetised animal there is a progressive fall in the arterial blood pressure. (The detailed analysis of the Kymograph tracings will be found opposite each in Volume II.) The heart beats more rapidly but the size of the corresponding manometer excursions slowly diminishes. The respiration becomes more rapid; the volume exchange with each respiratory act is increased, - the breathing is not only more rapid but fuller. In the severe forms the blood pressure reaches in about $1\frac{1}{2}$ minutes a minimum of 20-30 m.m. of mercury; the breathing thereupon, suddenly stops, the blood pressure rises some 10 m.m. during the succeeding $\frac{1}{2}$ minute - apparently an asphyxial rise, then to the accompaniment of intermittent gasping respirations gradually falls to the null-point and the heart ceases to beat (see Kymograph tracing Vol. II. p. 36).

In the less severe forms where recovery occurs there is a similar fall of blood pressure which, however, advances more slowly and seldom reaches the same degree as in the fatal cases. The minimum may not be reached till some 8-10 minutes after/

after injection corresponding to the period of maximum depression clinically. Thereafter the pressure rises fairly rapidly to reach in 4-5 minutes, something slightly under its original height.

SPLANCHNIC CONGESTION.

To record graphically the swelling of the abdomen which is so marked clinically is less easy. Plethysmograph tracings with a loop of intestine in a closed vessel full of Ringer's fluid show, however, that simultaneous with the fall of the arterial blood pressure there is a rise in the intestinal volume. (see tracing Vol. II. p.35).

(There are considerable mechanical difficulties. An air plethysmograph was unsatisfactory; the loop of intestine, despite all care to surround it with warm saline swabs, rapidly became deeply congested from the contact with the air. The fluid plethysmograph which surmounts this difficulty and records the pulse waves excellently, is very difficult to keep from leaking as soon as the increase in the volume of the enclosed intestine alters the level of the fluid in the recording and enclosing chambers).

Post mortem, it will be remembered, there appears an extreme congestion of the splanchnic area corresponding to this; microscopically as has just been shown in the histological description, the venules and capillaries of the intestine, especially in the intestinal villi, are tensely distended with blood; scattered/

scattered haemorrhages occur. In the omentum too, a condition of extreme congestion is present; microscopically capillaries full of blood appear where none are perceptible in the normal tissue, almost every corner in the connective tissue network enclosing the fat cells appears as a blood-containing capillary vessel.

IMMEDIATE CAUSE OF THE FALL OF BLOOD PRESSURE.

There are two immediate explanations possible for the concurrence of these phenomena. Presuming that the fall of blood pressure is due to a muscular paralysis of the arterioles (as held by Biedl and Kraus), the full effect of the heart beat comes directly on the capillary circulation and may be conceived as distending these vessels and the venules to the point of rupture. Against this, however, there are certain considerations. Raising the blood pressure by stimulating the arterioles with barium chloride does not prevent the cycle of symptoms - unlike the case of the dog - nor has it any perceptible therapeutic effect on the symptoms. Adrenalin too, while it causes a temporary rise in the rabbits blood pressure - again unlike the anaphylactic dog - effects/

effects no improvement in the animal's condition. Further in nitrite or chloral poisoning where an extreme degree of arteriole-paralysis occurs the capillary engorgement and haemorrhages are absent (Schmiedeberg (90)). Alternatively the phenomena might owe their origin to the conditions of the capillary circulation. A large amount of evidence exists that the capillaries are capable of contraction and dilatation altogether independent of the pressure in the vessels behind them.

Roy and Graham Brown (86) in their classical experiments on the capillary circulation, clearly determined that the diameter of the capillaries in the frog was, within certain limits, entirely independent of the arterial blood pressure. More recently Steinach and Kahn (93) have demonstrated in mammals also an independent contractility of the capillary walls, while still more recently Heubner (44), in a research on the toxic action of the double salts of gold, comes to the conclusion that there exists a definite group of substances which produce symptoms purely in virtue of their toxic, paralyzing, action on the capillary endothelium.

(These will be referred to again). Such a hypothesis - that the normal tonus of the capillary walls is destroyed as a result of the anaphylactic assault - certainly/

certainly suffices to explain the symptoms and appearances described. (This capillary paralysis and consequent congestion is probably not confined to the splanchnic area; (cf. the preliminary blushing of the ears), but is most strongly evidenced there owing to the mechanical conditions (e.g. villi) permitting best such a vascular engorgement). On this assumption we see the blood from the arteries pouring itself into a system of tubes whose total capacity has been enormously increased; (cf. the marked increase in intestinal volume); the animal is rapidly ensanguined by a process of bleeding into its capillaries and the blood pressure accordingly falls.

Whether there is any arteriole paralysis in addition is immaterial; the type of the kymograph tracing is certainly against this; the pulse waves instead of increasing in amplitude with the fall of pressure (as tends to occur in nitrite poisoning), become smaller and smaller exactly as in an animal which is being bled to death.

THE RESPIRATORY PHENOMENA.

This then may be the immediate explanation of the fall of blood pressure. To what is the pronounced respiratory character of the death due? The fall/

fall of blood pressure alone might conceivably produce an anaemia of the medullary centre sufficient to account for it. But in nitrite poisoning a greater depression may occur without cessation of respiration; further, artificial respiration, however prolonged, has never resuscitated anaphylactic animals. The clue is, I think, to be found in the lung condition (fully described in the histological section). There is present a quite peculiar thickening of the alveolar walls; the capillaries are no longer visible between the greatly swollen endothelial cells; there is considerable distension in the branches of the pulmonary veins, and occasionally haemorrhage from these. The bearing of this lung condition on the respiratory arrest is, however, not clear; there is a certain amount of presumptive evidence - e.g. extremely venous nature of the arterial blood of anaphylactic animals at a stage when respiration is apparently vigorous - which inclines one to the hypothesis that the gaseous interchange in these abnormal alveoli is interfered with, - (cf. also the experiments on respiratory exchange); the respiratory centre after the period of excitation has passed is in consequence inhibited either by an excess of carbonic acid or by a deficiency of oxygen. The muscular weakness which is so marked a feature of the anaphylactic/

anaphylactic illness may depend to a large extent on the low blood pressure; some primary interference with the muscle-cells cannot be excluded however, as will be seen later.

THE MECHANISM OF THE SYMPTOMS.

B. IN THE GUINEAPIG.

(The only communication dealing with this is very recent and highly important. In March 1910 Auer and Lewis (4) published an experimental explanation of the immediate death in anaphylactic guineapigs. The lung condition they say is entirely responsible for the fatal result. In the tracing of the volume of the respiratory exchanges it is seen that the volume of air expelled from the chest becomes rapidly less and less at the end of the 3-5 minutes that precede death; respiration ceases with the lungs fully expanded and the most violent expiratory efforts failing to expel the air. The lungs post mortem, as has been mentioned, do not collapse when the chest is opened but remain in the condition of extreme distension (Illustration Vol. II.Pl.III). (This is not so in the rabbit where they collapse practically to the normal extent). Tetanic contraction of the muscular wall of the bronchioles is responsible and this develops as well in a pithed animal (under artificial respiration) hence being entirely of peripheral origin).

My own experiments in this direction had been begun but had not reached further than to establish the difference in the process from that in the rabbit when Auer and Lewis' paper appeared. I can, however, entirely confirm their results (see tracing Vol. II.Pl.XVIII). Still more recently Biedl and Kraus (13) have published a paper in which they come to precisely the same conclusions as Auer and Lewis.

The blood pressure in such a guineapig does/

does not fall until after the respiration has definitely ceased; on the contrary there is after injection a slight rise, in all probability an asphyxial manifestation.

In the milder degrees of anaphylaxis no definite kymographic manifestations are obtainable. These correspond to the cases where clinically a violent pruritus is the chief symptom. The anaesthetic veiling this undoubtedly gave rise to Besredka's (10) original statement that ether anaesthesia was a perfect antidote to the anaphylactic illness. (Rosenau and Anderson (85) have already pointed out that death with the usual pathological appearances certainly occur under ether anaesthesia, the clinical symptoms being merely masked by it.)

THE RESPIRATORY EXCHANGE AND BODY TEMPERATURE.

A. IN THE GUINEAPIG.

There is, however, a definite stage of depression observable in which the guineapig, the irritation manifestations past, crouches in a corner or lies at full length - a position practically never found in the normal animal. If disturbed, its movements are feeble and the whole animal feels limp and/

and flaccid. The temperature is markedly subnormal. It is difficult to bring this condition to objective demonstration but an expression for it may be found in the figures for the respiratory exchange.

(The experiments on this point were performed with an apparatus constructed by Dr. T.S. McIntosh, the description of which is shortly to be published. It consists of a glass respiratory chamber through which air from the exterior is drawn by means of a water pump at a rate measured accurately by means of a large gas meter. Samples of the outgoing air are taken at a uniform rate over a definite period - usually about 40cc. of breathed air was collected during 30 minutes - and analysed in the Haldane's gas-analysis apparatus. The percentage composition of atmospheric air being known, that of the respired air corresponds to a certain volume-addition of carbonic acid gas and a certain volume-abstraction of oxygen (a correction based upon the nitrogen percentage eliminates the error due to the fact that the oxygen absorption is greater than corresponds to the carbonic acid output).

The volume of respired air as read from the meter is reduced to normal temperature and pressure and the actual output of carbonic acid and absorption/

absorption of oxygen in cc. calculated then directly. This apparatus presents certain advantages over the usual weighing method and with it excellently concordant results are obtained.

(I am deeply indebted to Dr. McIntosh for the permission to use it and for the painstaking instruction he afforded me in acquiring the necessary technique). The steps in the calculation are shown in the experiment which follows.

Sensitive guineapig (series 4, white), 540 grams, fasting 30 hours, put in respiratory chamber at 10 a.m. Rectal $T_o = 101.2^\circ F$; Sample begun 10.33 a.m., meter reads 0.7 cft; sample ends 11.3 a.m., meter 3.75 cft. Air $T_o = 14^\circ C$. Barometer 732 m.m.

Analysis readings: Volume of Sample = 20.042 cc.

After removing $C O_2 = 19.978 = .064 C O_2$

" " oxygen = 15.890 = 4.088 oxy-
gen.

$.064 cc. C O_2$ in 20.042 cc. air = $\frac{.064 \times 100}{20.042} =$
3.18 %

Factor for correction of burette = $1.008 \times 3.18 =$
 $.32 \% C O_2$

4.088 in 20.042 cc. air = $20.397 \times 1.008 =$
20.56 % oxygen.

Calculation for correction of volume for oxygen percentage:

100cc. - (20.56 + .32) = 79.12 - If 79.04
(= nitrogen percentage in atmospheric air)
corresponds to 20.93 of oxygen, to what
amount of oxygen does 79.12 correspond?

$\frac{20.93 \times 79.12}{79.04} = 20.95$

.32 % of $C O_2$ minus .03 % ($C O_2$ in atmospheric air) = .29 % $C O_2$ added; 20.56 subtracted from 20.95 (= corrected percentage) = .39 % oxygen abstracted.

Respiratory quotient = $\frac{29}{39} = .73$.

Volume/

Volume of air thus affected = 3.05 cft. in 30 minutes.

Corrected to 0°C and 760 m.m. = 156 litres per hour.

Guineapig (say) $\frac{1}{2}$ kilo. produced per hour per kilo
body-weight.

and used up $156 \times 2.9 \times 2 = 904$ cc. C O₂
 $156 \times 3.9 \times 2 = 1216$ cc. oxygen.

The further course of the experiment may now be put
in tabular form.

QUOTIENT	LITRES N.T.P. per hr.	VOLUME per kilo C O ₂	PER HOUR. OXYGEN.
Before injection 10.33-11.3 a.m. $\frac{29}{39} = .73$ T° = 101.2 F: placid.	156	904 cc.	1216 cc.

(11.58 a.m. - Injection 5cc. horse-serum intraperiton
eal).

After injection 12.12-12.42 p.m. $\frac{27}{40} = .67$ T° = 95.5°F: severe symptoms.	150	824 cc.	1200 cc.
1.25-1.35 p.m. T° = 89°F. extremely prostrate. $\frac{15}{15} = 1.0$	153	456 cc.	456 cc.

Total percentage drop in C O₂ production = 46 %.

An example where the symptoms were slight
(indeed confined to restlessness and scratching) may
be given.

Sensitive guineapig (brown series 4) 560 gram, fast-
ing 24 hours, put in respiratory chamber
at 11.45 a.m.

QUOTIENT	LITRES N.T.P. per hr.	PER KILO C O ₂	PER HOUR OXYGEN
Before injection 12.15-12.45 p.m. $\frac{55}{61} = .9$ $T^{\circ} = 100.7^{\circ}\text{F}$: quiet.	137	1367 cc.	1516 cc.

(Injection 5 cc. horse-serum intraperitoneal at 1 p.m.)

After injection 2-2.30 p.m. $\frac{81}{83} = .97$ $T^{\circ} = 97.8^{\circ}\text{F}$: still scratching.	99	1458 cc.	1510 cc.
4-4.30 p.m. now quiet: depressed $\frac{37}{47} = .79$ looking $T^{\circ} = 97.$ 0°F .	167	1125 cc.	1392 cc.

Percentage drop in C O₂ production = 18 %.
(The mean error of experiment in these results is certainly under 5 %.)

In three other sensitive guineapigs in which the respiratory exchange was estimated before and after assault the percentage drop in C O₂ was respectively 19 %, 27 %, and 17 %, three hours after injection.

A CONDITION OF DEPRESSED METABOLISM RESPONSIBLE.

The Rectal temperature dropped in that time on an average 4°F , (thereafter rising to the original point or not infrequently a little over). This fall/

fall in the body temperature cannot depend to any marked extent on interference with the heat regulating mechanism, i.e. on an increased heat loss, but must be due chiefly to the diminution in heat production which is expressed by the notable decrease in CO_2 excretion. There must exist, therefore, as a result of the anaphylactic shock a condition of depressed metabolism more or less prolonged. Whether this is confined to the muscular tissue in which it shows itself by the flaccidity and loss of tonus mentioned - or extends to all the body-cells, is difficult to determine experimentally. Some theoretical considerations will be submitted later.

B. IN THE RABBIT.

In the rabbit the state of affairs is very similar. Seven experiments have been performed.

The protocol of one of these may be submitted in detail as it illustrates the interesting condition of passive production of the symptoms.

Black + ⁰ (young, fresh rabbit) weight 900 grams, fasting 20 hours.

10.20 a.m. intravenous injection of 9 cc. inactivated rabbit serum.

(This serum contained a strong precipitin ∇ horse).

10.25 a.m. put in respiratory chamber: no symptoms.

10.40-11.10 a.m. Sample of respired air collected:
showed

per kilo per hour 990 cc. CO_2 produced.

1106cc. oxygen absorbed.

11.25/

11.25 a.m. intravenous injection of 5cc. horse-serum: put at once in respiratory chamber: symptoms definite, slight but obvious prostration: severe panting: marked looseness of bowels.

11.45 a.m. apparently recovered: still breathing a little heavily.

12.9-12.39 p.m. Sample of respired air taken: showed:
per kilo per hour 646 cc. C O_2 produced
940 cc. oxygen absorbed.

A drop of 35 % in the C O_2 production.

Control Experiment:

Black white collar $\frac{0}{1}$ (young, fresh rabbit) weight
950 grams fasting 20 hours.

12.58 p.m. received intravenous 8cc. inactivated rabbit serum.
(This serum from a normal rabbit).

1.2 p.m. put in respiratory chamber: no symptoms.

1.30-2 p.m. sample of respired air collected: showed:
per kilo per hour 1092cc. C O_2 produced.
1107cc. Oxygen absorbed.

2.12 p.m. 5cc. horse-serum intravenous: put at once in respiratory chamber: no symptoms.

2.35-3.5 p.m. Sample of respired air collected: showed:
per kilo per hour 1008cc. C O_2 production
1093cc. oxygen absorption.

A drop of about 8 % only (cf. mean error of experiment 5 %). There is thus as the result of the interaction of precipitin and antigen in the circulation of a fresh animal a pronounced disturbance of the economy; this shows that the symptoms of anaphylaxis, in the rabbit at least, do not depend entirely on a peculiar condition of certain body-cells. The results of other experiments may be summarised in the subjoined table.

RABBIT/

RABBIT	PRECIPITIN AND SYMPTOMS	PERIOD	PER KILO G O ₂	PER HOUR. OXYGEN
Grey ♂ 1370 gram (fast- ing)	moderate precipitin	$\frac{1}{2}$ hour before injec- tion	753 cc.	823 cc.
received 2cc horse-serum as assault- ing dose.	consider- able pros- tration.	$\frac{1}{2}$ -1 hr. after injec- tion.	575 cc.	672 cc.
12 days later.	now recov- ered: some- what lan- guid.	2 $\frac{1}{2}$ -3 hours after injec- tion	559 cc. = 26 % diminu- tion.	687 cc.
4 hours af- ter, re- ceived 5 cc. horse-serum intravenous.	no symp- tom: 're- fractory'	4 hrs. after first injec- tion. $\frac{1}{2}$ hr. af- ter 2nd.	601 cc.	965 cc.
now not fasting	normal	40 hrs. after	950 cc.	1050 cc.
B. & W. Russian ♂ 2380g fast- ing.	very weak precipitin	$\frac{1}{2}$ hr. before injec- tion.	726 cc.	801 cc.
received 2cc. horse- serum as assaulting dose.	very slight symptoms: some pant- ing.	$\frac{1}{2}$ -1 hr. after injec- tion.	702 cc. = prac- tically no fall	755 cc.

Black/

Used 16 days before for passive anaphylaxis
experiment recorded 7.1.26

RABBIT	PRECIPITIN AND SYMPTOMS.	PERIOD	PER KILO C O ₂	PER HR. OXYGEN.
Black ⁰ young: +950 grams fast- ing. *	very slight precipitin.	$\frac{1}{2}$ hour before injec- tion.	673 cc.	805cc.
received 4cc. horse-serum intravenous.	slight de- pression only.	$\frac{1}{2}$ -1 hr. after injec- tion.	609 cc. = 10 % fall.	839cc.
Same animal 13 days later.	weak pre- cipitin.	$\frac{1}{2}$ hour before injec- tion.	654 cc.	777cc.
received 4 cc. horse- serum in- travenous.	some pro- stration for 15 minutes.	$\frac{1}{2}$ hour after injec- tion.	523 cc. = 20 % fall.	543cc.
-	now re- covered.	1-1 $\frac{1}{2}$ hours after injec- tion.	654 cc.	722cc.
Himalaya ⁰ 1700 gram. fasting: last dose 15 days before.	moderate precipitin	1 hour before injec- tion.	621 cc.	728cc.
5 cc. horse- serum: in- travenous.	consider- able de- pression.	$\frac{1}{4}$ - $\frac{3}{4}$ hr. after injec- tion.	507 cc. = 19 % fall.	645cc.

* Last used 16 days before for passive anaphylaxis
experiment recorded p.106 .

THE GENERAL PROBLEM OF ANAPHYLAXIS.

RABBIT	PRECIPITIN AND SYMPTOMS.	PERIOD	PER KILO C O ₂	PER HR. OXYGEN
	recovered apparently.	2-2½ hours after injec- tion.	520 cc.	680 cc.

THE BODY TEMPERATURE.

The fall of the temperature is decidedly less marked in the rabbit than in the guineapig but equally constant. An average fall of from 1° - 2°F. at the end of ½ hour occurs where the symptoms have been at all pronounced; there is thereafter a rapid return to the previous level or a little above it.

The first description of the anaphylactic syndrome proper is that of Richet (75) in 1902, the commencement of a long series of researches in which THE/ he was appreciating the nature of the phenomenon and conferring on it the name, he lays down the laws which it appears to obey. (Even before that date

THE GENERAL PROBLEM OF ANAPHYLAXIS.

(Certain forms of increased susceptibility must be classed apart from the general problem. First discovered by V. Behring (6) the heightened susceptibility to Diphtheria toxin acquired in the course of immunisation has been a much quoted example. One of V. Behring's 'immune' horses died after a dose of toxin which injected into a fresh horse required not more than 1 c.c. of the former animal's serum to render it completely harmless. Similarly Brieger (16) describes the case of a goat which died of typical tetanus after a dose of tetanus toxin abundantly capable of neutralisation by its serum. V. Behring and Kitashima (7) in an experimental study of this question showed that guineapigs might be killed by a total quantity of Diphtheria toxin equivalent to $\frac{1}{400}$ of the M.L.D. when this was divided into minute doses given over a period of 17 days. Explanations for this phenomenon exist in terms of the 'side-chain' theory of Ehrlich (see Wassermann (96).)

The differences necessitating a different category for these cases are fundamental. They are (1) the fact that the death occurs with the typical toxin effects and not with the anaphylactic syndrome described (2) the fact that the serum of the sensitive animal does not transmit sensitiveness but a high degree of immunity to the fresh animal. Another separate form of supersensitisation is that to Cocaine described by Aducco (1.) and to Apomorphine by Richet (80) both in any case somewhat doubtful as regards the essential fact.

The natural hypersusceptibility to certain drugs e.g. Quinine. Salicine Iodides is also certainly entirely different in mechanism.)

The first description of the anaphylactic syndrome proper is that of Richet (79) in 1902, the commencement of a long series of researches in which besides appreciating the nature of the phenomenon and conferring on it the name, he lays down the laws which it appears to obey. (Even before that date in/

in conjunction with Héricourt (43), Richet had observed the death of rabbits in the course of immunisation with eel's serum. This observation like that of Flexner (29) with dog's serum had received little attention.) The experiments of Richet have been conducted with extracts from the tentacles of starfishes and the bodies of mussels (79.). From both of these a highly toxic albuminoid substance may be extracted, from the former Actinocongestine, from the latter Mytilo-congestine. (82.). The mode of preparation is important since by its use similar bodies of great or small toxicity have been prepared from a large variety of native proteins. A watery extract is precipitated by a large bulk of Alcohol (90%): the precipitate extracted with water and this second extract reprecipitated in the same way: the poison, a substance still giving the albuminoid reactions, is purified by several repetitions of this process, it being soluble in water but insoluble in strong alcohol.

In this way from the sponge 'Suberitis domuncula' a poison 'Suberitine' (81.) and from the plant 'Hura Crepitans' a poison 'Crepitine' () has been prepared. All these possess a common pharmacological action producing in dogs vomiting, depression, /

depression, and bloody diarrhoea with a fall of blood pressure and extreme congestion of the Gastro-Intestinal tract. All display strongly a sensitising action; for example the M.L.D. of actino-congestine with the primary dose is .004 gram per Kilo (producing death after several hours); a dose of .002 gram to an animal which received three weeks before a sublethal dose, is fatal within half an hour with the most acute symptoms of gastrointestinal irritation. The hypersensitive condition (which is definitely specific) persists for about seven weeks to give place to a certain degree of immunity. Passive transference is possible and even an 'in vitro' action of the sensitive serum on the toxin (vastly increasing its toxicity) is demonstrated ().

Closely similar to these poisons of Richet, is Papaine with which Pozerski (77) has produced in the guineapig most marked anaphylaxis the clinical and pathological picture being one of extreme gastrointestinal irritation.

Biochemical data regarding the changes in animals rendered sensitive to and assaulted by these substances are entirely lacking. Dogs only have been the subjects of experiment with very few exceptions.

THE ANAPHYLACTIC SYNDROME RESEMBLES
 THE PEPTONE EFFECT.

On comparing the symptoms described by Richet for these anaphylactic poisons with those produced by Biedl and Kraus on repeated injection of Horse and Ox Serum the strong similarity is evident. Not only so, but these symptoms, as pointed out by the latter observers, run remarkably parallel to the well-known Peptone effect on dogs. In this as in the Serum Anaphylaxis of these animals there is a prolonged fall of arterial blood pressure; the animals become comatose with swollen abdomen and evacuation of faeces; during this condition the blood is incoagulable - the Peptone plasma is got and there is a marked leucopenia; on recovery the animals are refractory for many hours to a repetition of the injection. All these phenomena appear with the serum anaphylaxis, and, more striking still, subjection to either this or the peptone effect renders the animal refractory to an injection with the other. It would seem that an explanation might lie to hand: the animal's serum has acquired as a result of the primary dose of serum the capacity to break down to a peptone body the foreign protein introduced/

introduced and hence set up the usual peptone effect. Direct testimony on this point however does not exist. A similar explanation for the anaphylactic poisons of Richet is of course met by the difficulty of the minute dose. Whereas for the full peptone effect .3 gram of Peptone per kilo body weight is necessary an illness much more severe may be set up by .055 gram per kilo of these. This is not an insuperable obstacle however. According to Popielski (74) the effective constituent of peptone is a substance present in extremely minute quantity and quite independent of the proteoses and peptones. This substance is thought to be a constant constituent of protein but only set free and capable of inducing its action after peptic digestion; (products of Tryptic digestion have not this action as was long ago shown by Fano (27).

(The peptone syndrome first described by Schmidt-Muhlheim (89) has been produced by a great variety of organic substances many of which recall the action and mytilo congestive origin. For example Delezenne(23) with eel's serum and extracts of its organs and Pick and Spiro (69) with the serum of other murenidae and with the liver of crustacea produced in dogs the typical incoagulability of the blood/

blood and the symptoms of peptone poisoning.)
the rabbit and dog is sufficiently obvious.

THE COMMON FACTOR.

The question of some factor common to these various agents is apparent and of the first importance. It must be remembered that in both the rabbit and the guineapig the peptone effect cannot be induced. In the former no symptoms are produced until relatively enormous (rapidly fatal) doses have been given (1 gram per kilo) even then blood coagulation is only slightly delayed. (Fano (loc.cit)). My own experiments show that instead of leucopenia there is a remarkable immediate leucocytosis on injecting doses of .3 gram of peptone per kilo intravenous, the count changing in the course of a few minutes from say 10,000 per c.m. to 25,000. Such a dose in the normal rabbit produces a very temporary fall of blood pressure apparent on the kymograph but hardly clinically. In the anaphylactic animal during or shortly after the stage of depression, such a dose of peptone produces a severe relapse or reappearance of the symptoms: there is apparently a superposition possible of the two effects. (The leucocytosis occurs here also; the leucocytes which as the result of the anaphylactic shock had fallen to 3,000 per c.m. rise/

rise at once to 10,000 or 12,000.) The contrast in the rabbit and dog is sufficiently obvious.

In the guineapig according to Persano (67) doses of .6 gram per kilo have no apparent effect (in the recently fed animal) but a slight excess over this produced rapid death: (in the fasting animal a slight delay in the blood coagulation may be induced with these doses.)

In the rabbit however (and to a less obvious extent in the guineapig) the actual anaphylactic syndrome much resembles the peptone effect of the dog although peptone itself is incapable of producing this.

There is however, another symptom-complex still which bears a resemblance to anaphylaxis in the rabbit. In surgical shock it is well known that there is a condition of low blood pressure not responsive to adrenalin and referable to splanchnic congestion while in a paper by Rüchel and Spitta (87) (on the behaviour of the leucocytes during blood coagulation), a similar effect is described on section of the splanchnic nerves. Besides a marked fall of blood pressure with congestion of the portal system there is a pronounced leucopenia, the count falling from 12,000 to 3,000 per c.m.

THE VASCULAR ENDOTHELIUM.

Putting together then all these facts it seems possible to get a little nearer to the problem. The vascular conditions of the portal area are normally presided over by the splanchnic sympathetic nerves: these being cut an immediate loss of tone results not only in the arterioles but in the capillaries as shown by Steinach and Kahn (93). In consequence there is a pronounced stagnation of blood (occurring with readiness and to a greater degree here in the abdomen than elsewhere owing to the physical conditions .) To this blood stagnation the leucopenia is to be ascribed; the most responsive leucocytes, the polymorphs, settle quickly out of the slowly circulating blood, in particular perhaps in the liver, leaving the systemic circulation relatively poor.

Finally that blood poor in leucocytes coagulates less vigorously is a phenomenon widely observed. But the interference with vascular tone may occur peripherally instead of centrally. With splanchnic nerves intact and transmitting stimuli there may be no response and a continuous absence of tonus as a result of a direct interference with the vascular tissue itself; an extreme depression of metabolism/

metabolism in the endothelial cells which build up the capillaries rendering them temporarily equivalent to dead cells and producing complete abolition of the normal capillary tonus, would equally well produce the splanchnic stagnation and the blood picture directly consequent. The existence of such a condition has already been deduced from the experimental analysis; the histological picture also agrees. In the rabbit then the root condition is disturbance of the capillary endothelium.

The mechanism in the dog seems certainly somewhat different, but the differences are by no means fundamental. It would seem that the liver of a sensitive dog reacts to the appropriate serum as the normal liver does to peptone by discharging into the circulation these peculiar, probably acid, Antithrombin substances found in the peptone plasma; these or others produce a depression of vascular tonus in the portal system, probably by direct action on the endothelial cells (the coexistence of delayed blood coagulation and the tendency to capillary haemorrhages in human pathology is significant on this point). Adrenalin and sympathetic stimulation are therefore both without effect while the Barium effect may as reasonably be referred to an action on the capillary tonus as on the muscle which has failed to respond to so powerful a stimulus as that of adrenalin/

ardenalin. is less prominent. The symptoms (pruritus)

[In the rabbit the liver does not act in this way; not till the peptone reaches a concentration which directly injures the cells of the capillary endothelium, evidenced by multiple capillary haemorrhages, does it produce symptoms.]

It is of interest to note in this connection that the substances which we have seen to produce primarily a syndrome resembling that found in anaphylaxis come into the category of those substances which Heidenhain (cit. Ellinger (26)) called 'Lymphagogues, Class I'. These according to Heidenhain produce effects which can only be explained by a direct injury or stimulus to the capillary endothelium. He mentions extracts from mussels (cf. mytilocongestine), organ extracts, peptone and bacterial decomposition products (cf. Sepsin, Faust (28)).

Along with these substances may be classed the fatigue-toxin or Keno-toxin of Weichardt this substance, originally an extract of the muscles of fatigued animals, latterly prepared from a great variety of proteins by somewhat ill-defined means and found also in the urine after fatigue, produces a set of symptoms of which muscular weakness and coma appear to be the most definite. On the relationship with physiological fatigue no compelling evidence exists.

'Urchypotensine' cf. Abelous and Bardier () is probably of the same nature: it produces a prolonged fall of blood pressure and is said to produce anaphylaxis. Compare also Pearce ().

In the guineapig the splanchnic cycle of phenomena/

phenomena is less prominent. The symptoms (pruritus) suggest distinctly a disturbance of the capillary endothelium in the skin but the most striking action is on the plain muscle of the respiratory tract. It is an open question however, whether the bronchial spasm is primary, or secondary to an irritative condition of the cells lining the air passages, including the respiratory endothelium.

We see then that the production of the same peculiar group of symptoms by substances of very different origin may be explained by the hypothesis of a common action on the capillary endothelium. Why should this action appear on repeated parenteral injection of a foreign protein in itself harmless?

THE CAUSE OF ANAPHYLAXIS.

A. THE ENDOTOXIN THEORY.

In the case of the acquired toxic property of fixed cells as blood corpuscles, organ-emulsions, bacteria, two modes have been demonstrated, immediate and delayed, by which death may be produced. These are sufficiently different to require an absolute separation: the first, immediate, mode is the anaphylactic/

anaphylactic syndrome proper, with which we have been chiefly concerned: the second or delayed action has hitherto not been sharply differentiated. Superficially regarded a simple explanation for the latter lies to hand. In the case of red blood corpuscles, for example, it has been shown by Gottlieb and Lefmann (39) that certain soluble constituents of the erythrocyte stroma possess a definite toxic activity. In the treated animal as a result of the primary sensitising dose a lytic substance, as is well known, is developed. The animal has acquired in consequence the power to liberate rapidly in its circulation their toxic constituents from the corpuscles of the assaulting dose. (The solution that takes place shows itself both 'in vitro' and in 'vivo' by the presence of Haemoglobin)

(On the nature of these constituents there is little but conjecture; they are doubtless lipoids and, like many of these, cell-poisons. On their toxicology, too, data are almost entirely lacking: a profound alteration in the chemistry of the body cells under their influence is indicated by the staining reactions post mortem.)

The conditions with other cells are similar; the acquired capacity of rapid solution, of value presumably as a defence against natural invasion by these (e.g. bacteria), becomes under the experimental conditions defined a disastrous property; the products of solution, of a toxicity varying with different/

different cells, thus rapidly liberated, fatally upset the animal's economy.

This line of reasoning has been followed especially by Wolff (99) and is a direct development of the 'endotoxin' theory of Pfeiffer put forward to account for the 'sterile death' in guineapigs receiving large doses of *V. Cholerae* in the peritoneum.

M. Nicolle (63) adopts a modification of this endotoxin theory. In the course of a far-reaching generalisation on the behaviour of the animal economy towards foreign proteins, he finds it necessary to assume that two antagonistic processes must occur. With certain proteins (certain bacteria, extracellular toxins) a coagulation process predominates on contact with the serum of the immunised animal, followed by a lysis which is in consequence leisurely..

With other proteins and especially at certain definite intervals in the course of immunisation a lytic process is most prominent and to this the anaphylactic effect is due. This lytic process is not a gross one of cytolysis only but with proteins of all characters including toxins and serum-proteins a solution of the molecule takes place and the toxic component/

component formerly harmless by reason of its fixation in the large molecule is set free. According as the coagulins, (in the widest sense, including true antitoxins,) or the lysins predominate in the reaction bodies of a treated animal so there will result immunity or supersensitiveness

(The objective evidence furnished is not convincing.)

B. THE FERMENT THEORY.

The theories of both Wolff and Nicolle attempt to apply to the hypersensitive condition towards infecting agents the theory originally put forward by Richet (loc. cit.) for the congestive poisons discovered by him. Richet's fundamental experiment was as follows. A dog received a dose of mytilic-congestine, sublethal but producing definite illness (.022g per kilo). Twenty days after, its serum (85 c.c.) is injected into a fresh dog which is two days later subjected to a large assaulting dose of the mytilic-congestive (.047 gram per kilo). In contrast to control fresh animals which receive nearly twice this dose of the poison and survive, the passively sensitised dog has immediate severe symptoms and dies. The sensitive serum itself is harmless; the/

the dose of poison alone is quite insufficient to cause death; the combination of the two is fatal. The analogy of the genesis of Hydrocyanic acid being followed -- (Amygdalin alone = harmless the ferment emulsin alone = harmless, the combination producing a violent poison), -- a ferment 'toxogenine' is assumed to be present in the sensitive animal's serum. This reacts with the mytilo-congestine to produce a poison acting immediately and violently instead of slowly and mildly.

(The passive transference, particularly when the combination of sensitive serum and congestine is conducted *in vitro* is by no means of regular occurrence. Of six experiments for example only one was successful).

Anaphylaxis to Richet means 'Toxine + Toxogenine = Apotoxine'. 'Apotoxine' is the poison of the anaphylactic syndrome.

Vaughan and Wheeler (95) describe experiments with egg-albumin in which they break up the protein molecule (by decoction with alcohol and strong alkali) into poisonous and non poisonous fractions. They ascribe anaphylaxis to the same process: in the sensitized animal the cells possess a 'Zymogen' which, on coming in contact with fresh supplies of the protein which stimulated its formation, is/

is rendered active and liberates rapidly the toxic fraction.

Heilner (41) and Salus (88) also assume a ferment action in the supersensitive state, the former as the result of metabolism experiments with foreign proteins parenterally introduced (-an increased capacity to assimilate these is demonstrable), the latter by inductive reasoning from the clinical conditions of serum sickness in man.

Friedemann and Isaac (34.) at the end of their papers on metabolism of parenterally administered protein explain hypersensitive phenomena as the expression of an explosive production of digestion products on contact of the assaulting dose with the cells and their juices.

V. Pirquet and Shick (71.) express a somewhat similar opinion though in a much more cautious and general form. They again refer especially to the serum sickness of man. (V. later)

G. THEORIES FOR THE IMMEDIATE ANAPHYLACTIC SYNDROME

(with a statement of the chief experiments
on which they are based)

The/

The theories mentioned so far, though they may be fair approximations to a general explanation for the conditions underlying the late sequelae of anaphylaxis, especially the interference with metabolism, meet at once with difficulties on being applied to the phenomena of immediate anaphylaxis, with which we are chiefly concerned.

(One difficulty, (from which however there are several ways of escape open) is the absence of any demonstrable poison in the serum of the suffering animals. Serum from a guineapig dying with the Theobald Smith symptoms may be given to a fresh animal to any amount without causing symptoms. It is the same with a rabbit. I have given intravenously to a fresh rabbit of 1200 grams no less than 35 c.c. of the serum of an animal dying acutely as a result of an assaulting dose; yet under these conditions no symptoms appeared). Even in the case of the anaphylaxis to bacteria and other cells, (where a lytic substance is actually demonstrable in the sensitive animal's blood) the endotoxin and ferment theories fail. Delancé (22) has made detailed studies in the case of the guineapig and comes to the conclusion that the immediate anaphylaxis to bacteria and blood corpuscles cannot depend (a) on the agglutinin/

agglutinin developed (the time relations negative this) (b) on the lysin (a great degree of lysis may occur without symptoms: after symptoms are past, with a lysin as strong as before, no further symptoms can be induced).

My own experiments on the rabbit similarly show (a) that the absolute lytic strength of its serum is no criterion of the susceptibility of the animal, and (b) the 'refractory' condition coexists with a lytic power as high as before. Further it is certainly doubtful whether any lytic power exists in the sensitive animal's serum for such substances as serum protein. There is none demonstrable biochemically and the incubation together of sensitive guineapig's serum with Horse Serum does not produce a toxic mixture for normal animals (Rosenau and Anderson (83) Otto (64). (In the rabbit this is not so, provided the precipitum is not removed before injection, c.f. Briot (17). The explanation of this difference necessitates a special discussion (see later).

The current theories for the immediate anaphylactic syndrome consequently take up a different stand point. They are mostly based on experiences with the guineapig and several of them are coloured by a view of the pathology of the symptoms now known to/

to be absolutely erroneous. Both Besredka (10) and Kraus and Doerr (24), namely, regarded the central point of the mechanism as lying in the nervous system: they were led to this by the marked convulsive movements in the acute death and the fact that general anaesthesia appeared to confer immunity as it was known to do for convulsive poisons as strychnine. Besredka (loc. cit.) too, employing intracerebral assaulting doses (the first to do so), found an extreme susceptibility existed, doses of $\frac{1}{100}$ c.c. being sufficient to cause death. (The nature of this death is still obscure: it is certainly distinct from the other forms).

BESREDKA'S THEORY

Besredka's theory may be said to hold the field at present. It depends largely however, on experiments by no means crucial; they are chiefly of a physico-chemical nature and bear upon the substances used on the one hand as sensitisers on the other as assaulting agents. He found that heat ($100^{\circ} - 120^{\circ}\text{C}$ for 15 minutes), gross coagulation being prevented by previous dilution with distilled water) did not prevent Horse Serum from sensitising guineapigs to the/

the intracerebral test. The assaulting capacity on the other hand, the so-called toxicity of the serum is definitely diminished by 20 minutes sojourn at 75°C and is completely removed by a short subjection to 100°C . Two substances are therefore postulated in Horse Serum a 'sensibilisinogene,' highly thermostable, which is responsible for the sensitive condition, and an 'antisensibilisin,' thermolabile, to which the phenomena on assault are due. A third body is necessary for the reaction, the 'sensibilisin': this is produced by the guineapig under the stimulus of the 'sensibilisinogene,' affixes itself by preference to the nerve cells and has a powerful affinity for the 'antisensibilisin' of Horse Serum. The anaphylactic syndrome is due to the sudden combination of sensibilisin and 'antisensibilisin' in the nervous system. If the rapidity of this combination is retarded and the sensibilisin removed from the nerve cells slowly instead of suddenly, no symptoms appear. Hence is explained the 'refractory' condition induced by a preliminary injection of very minute quantities of dilute Horse Serum, heated serum or serum denatured by alcohol (11). The refractory period which precedes the establishment of the sensitive condition, during which however sensibilisin is present/

present as shown by passive sensitisation - is explained by supposing that as long as Horse-Serum is still circulating in the blood the sensibilisin attaching itself to the nerve cells is continually being combined and rendered harmless. Anaesthesia permits the combination to occur in the nerve-cells without injuring these; that it has occurred is shown by the 'refractory' condition of the animal after waking. Even applying this theory to the intracerebral test only, the assumptions are too great. The assumption of different substances sensitising and combining is not established by the heating experiments: the great difference in magnitude between the sensitising and assaulting doses, - one millionth c.c. sufficing for the former while at least one hundredth is necessary for the latter, - must be considered. Denaturation of the protein just short of complete may still permit of sensitisation while there may be insufficient unaltered protein left to act as an assaulting dose: such an explanation is certainly as plausible as Besredka's. Finally it has been shown by Auer and Lewis (4, 79) that combination of sensibilisin and antisensibilisin can occur and the typical symptoms be produced in pithed animals i.e. altogether apart from the nervous system.

Thus/

Thus shorn of its special features Besredka's theory does not differ from the 'two substance' theories of Otto, V. Pirquet &c.,

OTTO'S THEORY

These postulate a single substance in the Horse Serum to which a single antibody is developed. This antibody or anaphylactic reaction body, is of quite peculiar sort and is not demonstrable in any sense except as effecting the anaphylactic illness. It withstands heating to 60°C . It is not neutralised by Horse serum either in vitro or, as is shown by the refractory animal's serum containing it, 'in vivo'. It requires to be well distributed through the body, (in passive transference), before effects can be produced (incubation period of 20 hours).

Symptoms are due to the liberation of a poison by it on meeting the assaulting dose of Horse Serum.

THE THEORY OF GAY AND SOUTHARD.

(1931) who produced an anaphylactic reaction by subcutaneous injection of Tuberculin in a normal individual two days after conjunctival administration of the same. The hypothesis of 'anaphylactin' exists. They deny the necessity of an antibody and locate the process responsible for the symptoms in the body cells generally. The peculiar conditions necessary for sensitisation (e.g., the minute dose and long incubation period), they explain by supposing an assimilable fraction to exist in the Horse Serum molecule. This substance, 'anaphylactin', persisting in the circulation, exerts a constant stimulus to the assimilating functions of the cells for Horse serum. There is in consequence when these are brought in contact with abundant Horse Serum such an overexertion of these cells in the attempt to assimilate that their metabolic processes are seriously interfered with and the symptoms produced.

Against Gay and Southard's hypothesis is the fact of passive transference by the serum: they explain this by assuming that this really effects an active sensitisation from the presence of unexcreted anaphylactin. The rapidity of such a sensitisation is certainly much greater than with Horse Serum alone but examples of such rapid sensitisation exist, as for/

for tuberculin (c.f. Calmette, Breton, and Petit (19.) who produced an ophthalmo-reaction by sub-cutaneous injection of Tuberculin in a normal individual two days after conjunctival adhibition of the same). The hypothesis of 'anaphylactin' explains well, however, the active sensitisation of fresh guineapigs effected by injection of the serum of animals which have long ago received a dose of Horse Serum.

For the Theobald Smith phenomenon then, this theory, though rather vague on the question of the mechanism, furnishes a plausible explanation.

THE THEORY OF INJURY TO THE ENDOTHELIAL CELLS.

DISCUSSION OF THE METHODS BY WHICH THIS

MAY BE PRODUCED.

But the conditions in the rabbit are not to be reconciled with these theories. To obtain a theory of general application to the different cases of anaphylaxis it is necessary to take up again the conclusion already reached regarding the mechanism of the symptoms, i.e., that injury to the cells of the capillary endothelium is the determining factor. How then is this injury brought about? The possible causes of injury may be classified as (1) changes in the/

the fluids bathing the cells or (2) direct action on the cell protoplasm.

I. CHANGES IN THE FLUIDS BATHING THE CELLS.

(A) Alteration in the density and osmotic tension of lymph and plasma may be readily conceived as at least temporarily injurious.

(Heilner (42) has suggested this as an explanation, reasoning from the effects of injection of large quantities of hypertonic salt solutions (4% Sodium chloride) on metabolism. He considers that the sensitive animal possesses blood fluids in a condition of unstable equilibrium so that any sudden addition, be it of antigen or merely of non-isotonic salt solution, produces an abrupt change in the osmotic relations with the cells and a consequent interference with the life processes in these. The conditions of his experiments - death produced in rabbits sensitive to Horse-serum by a subcutaneous injection of 200 c.c. of 4% salt solution, which is usually withstood by normal animals, - are hardly of a kind to sustain the theory unsupported. (Frey (32) could not reproduce the result in the sensitive guinea-pig but used much smaller quantities.)

Analogous observations exist however. In tuberculous animals injection of substances such as bouillon, salt solution &c., sets up a reaction closely resembling the tuberculin effect (Preisich and Heim (78)). In normal animals a preliminary injection of these substances sets up a reaction on subsequent injection of tuberculin (Kraus (48)).

Richet (80) observes that a previous injection of distilled water, saline &c., in sensitive dogs, though not producing any symptoms, modifies markedly the severity of a subsequent assault.

There is no direct evidence in existence of a change in the osmotic tension of the body fluids in anaphylactic animals. I have attempted experimental observations without concordant results. (Slight changes/

changes in specific gravity in the serum occur but not more than the addition of denser Horse-serum to the blood might account for, hence also in normal animals).

On the other hand the removal of an appreciable quantity of protein from the circulating plasma (i.e., the disappearance of the precipitin in anaphylactic rabbits) must affect the environment of the lining endothelium to some degree.

(B) The conditions of solution of the Calcium salts in the blood might be affected. The pharmacological action of Oxalates and Fluorides is suggestive. Both, but especially the latter, are powerful poisons producing a rapid fall of blood pressure with acute congestion of the abdominal viscera and death, as I have been able to assure myself, after a course of symptoms very like those in anaphylaxis. The animal receiving 0.1 gram of Sodium fluoride intravenous becomes rapidly limp with panting respiration and swelling of the abdomen; after 5-7 minutes the breathing stops, convulsions set in and death, with exophthalmos, swollen abdomen and heart still beating as in the anaphylactic animal, occurs.

Histologically the condition is an exaggeration of the anaphylactic congestion of the intestine/

intestine &c., extreme distension of capillaries with much haemorrhage. The lung condition of anaphylaxis is absent. The Blood pressure tracing resembles closely that in the anaphylactic rabbit.

(A cutaneous gangrene resembling the Arthus phenomenon may be produced by subcutaneous injection).

Precipitation of the lime salts is the cause assigned for this toxic action (Cushny (21)). It is conceivable that in the changes resulting from the sudden union of antigen and precipitable anti-serum an alteration in the complex conditions of calcium in the blood might arise.

Direct experiment, however, fails here again: the differences in the amount of Calcium (determined by incineration, exhaustion with hydrochloric acid, precipitation with oxalate, and weighing as oxide of Calcium) in the serum before and after a fatal anaphylactic illness are hardly outside the error limit.

Example: Serum before injection .0471% Calcium Oxide.

" after " .0395% " "

A greater effect on the heart's activity also ought to show itself if in the anaphylactic syndrome a Calcium/

Calcium-removal were at work.

In spite of the absence of direct evidence, however, the conditions of passive production of anaphylaxis in the rabbit force the conclusion that the occurrence of physico-chemical changes in the bathing fluids affects immediately the cells of the vascular channels.

If the conditions necessary for such reactions to take place in the circulation are provided, e.g., if first antigen (Horse Serum) is allowed to distribute itself (5 minutes), then antiserum (precipitin) injected into the circulation or vice versa, marked symptoms are produced immediately and an alteration in the physico-chemical condition appears, evidenced by the diminished lytic power of the fresh serum (complement).

The arguments in favour of the supposition that this change in complement is itself the responsible factor have already been brought forward (p.); the sole conclusion that they warrant is that, though the symptoms of anaphylaxis in the rabbit are associated with such a change in the serum, there is not proof that this is causally connected; the relation may equally plausible be the reverse, - the diminished complement may be the result of the cell injury.

injury. It is significant in this connection that the 'in vitro' complement-destroyers fail to produce complement diminution in corresponding dose 'in vivo'. This includes not only chemical agents such as fluorides, lecithin, metallic salts, &c., but diluted antigen. 'In vitro' 5 cc. of a 1% Dilution of Horse-Serum completely abolishes the complement activity of as much as 100 cc. of the sensitive (precipitin) serum, (i.e., more than the total serum in the body): this quantity injected intravenously produces neither symptoms nor a drop in the animal's complement strength.

Such behaviour certainly favours the latter hypothesis, that a cell-disturbance is responsible.

II. DIRECT ACTION ON THE CELL PROTOPLASM

There exist certain poisons, of chemical constitution widely apart, which have none of those affinities for the "noble" cells of the body (nerve, heart muscle, gland cells) possessed by most poisonous alkaloids and toxins of organic origin (such as snake venom, diphtheria and tetanus toxins). They act on the contrary simply as general protoplasmic poisons destroying

C O N C L U S I O N S.

The conclusions to be drawn from the available facts are:-

- (a) that the physico-chemical change in the circulating blood resulting from the interaction of precipitin and antigen and reflected by the diminution in complement may alone affect the capillary endothelium so as to produce modified but definite anaphylaxis (i.e. passive transference).
- (b) that the symptoms in actively induced anaphylaxis are at most only partially due to this change, the factor remaining being a direct action of the antigen on the cells of the capillary walls.

II. DIRECT ACTION ON THE CELL PROTOPLASM.

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destroying in high dilution for example those unicellular organisms which are quite insensitive to the former bodies. Injected intravenously they are prevented from acting at all on the 'noble' cells by being at once absorbed by the less highly specialised cells, which build up the circulatory channels. Certain of these poisons appear to have a particular affinity for the endothelial cells and are capable of acting on them by any channel of introduction.

All are characterised by producing a pathological picture closely resembling that of anaphylaxis; some act acutely like horse-serum in sensitive rabbits (e.g. gold-sodium-chloride, Emetine, Sepsine,) others produce the same symptoms and appearances more slowly and gradually (e.g. Arsenic, iron-sodium-citrate, tartarated antimony). I have been able to assure myself of this in the case of the double salts of gold and of iron, with emetine and with arsenic.

There is the same fall of blood pressure with congestion of the splanchnic area and cessation of respiration as the death signal, the heart continuing to beat, while post mortem there is capillary engorgement and haemorrhage in the organs. All these observations are on record in pharmacological literatures (gold salts vide Heubner (44), iron-salts v. Meyer and Williams (57), Arsenic v. Pistorius (72), Antimony/

Antimony v. Soloweitschyk (91), Emetine v. Podwyssotzki (73), Sepsine v. Faust (28),) but Heubner (loc.cit) is the first to draw attention to the common toxic action.

The typical syndrome can with most be elicited only by intravenous injection of doses large enough to be lethal within some minutes: if smaller doses are given at intervals, a total quantity much in excess of the lethal dose is borne without the typical symptoms - (later chronic symptoms may appear with certain of these). There must exist in fact a sufficient concentration of the poison in the circulation before the effect is produced on the endothelium just as in the sensitive rabbit where repeated small doses of antigen may be given without the least disturbance.

There is a similar leuccopenia, delayed coagulation and fall in complement as in anaphylaxis; the endothelial-cell poisoning is apparent.

On the immediate mechanism however no data exist; these substances injected intravenously disappear from the blood with extreme rapidity, presumably by combining with the cells, but no fine histological changes in these have been demonstrated. With the serum-anaphylaxis of the rabbit theoretical considerations/

considerations however point to a possible direct cause of injury. It is to be presumed that where in the blood plasma a large proportion of the protein is of a precipitable kind the cells bathed by it contain the same, or a closely similar, protein (without entering on the question of these as a probable source of the blood proteins). When therefore the antigen, e.g., Horse serum, comes in contact with them a portion of their protoplasm should be thrown suddenly into an insoluble non-functionating condition and the vital activities be proportionately hampered.

The skin reaction in sensitive rabbits supports this: a minute quantity of the antigen put just under the skin sets up within 24 hours a spot of deep erythema which is quite absent with fresh rabbits. (The Arthus phenomenon - ulcer formation after cedema and capillary haemorrhage - is also a supporting fact). These actions being entirely local most probably depend on the cells and not on an action through the medium of the fluids when at least a more diffuse effect might be expected. The conditions of the tuberculin skin reaction as will be mentioned later, also support the hypothesis. A recent observation in man is of great interest/

interest too. Stanculeanu and Nita (92) produced by conjunctival instillation in one eye a local sensitiveness to Horse-Serum, with acute inflammatory congestion on repeating the instillation; the other untouched eye with the same dose showed no reaction. The body fluids in this case cannot be the agents at work; the cells must be responsible. Unfortunately in the rabbit such local sensitisation does not occur so that this crucial experiment cannot be extended to it.

C O N C L U S I O N .

There is however sufficient evidence for the conclusion that the cells of the capillary endothelium in sensitive rabbits may be profoundly affected by application of circulating antigen, which probably acts somewhat as it does in serum by throwing a portion of the protoplasm out of action.

How does this apply to the Guineapig?

It is plain that this explanation cannot apply to the guineapig where no precipitin can be demonstrated and where no local reaction occurs. (Lewis (54) however records cases of guineapigs reacting to subcutaneous injections with oedema and ulcer formation: these were young animals).

Yet the conditions of passive anaphylaxis make/

make it evident that here the cells and not the body fluids are the chief reacting agents. Injection of sensitive serum followed by antigen has no effect unless a period of at least 20 hours is allowed to elapse between the injections, i.e., until the body cells have had time to absorb the substance conferring on them the power of reacting. The immediate production of anaphylaxis by transferring a precipitin serum is in striking contrast. It is necessary therefore to draw a sharp distinction between the form of reaction associated with precipitin and that where precipitin is absent although the symptoms of the reaction may be fundamentally the same. Much confusion exists in the literature from overlooking this (Cf. Doerr (25)) and conclusions on the nature of the anaphylactic reaction-body, drawn from the conditions of passive transference, are rendered hopelessly contradictory.

The guineapig does not naturally produce precipitin sufficient for the 'rabbit-type' of reaction: if by means of precipitin rabbit serum this is supplied, then the process may scarcely be distinguishable (Cf. tracing Vol. II. p. 43) from that in the rabbit, or may produce a typical Theobald-Smith form of death (Cf. Tracing Vol II p.). This however does not at all permit the conclusion, /

conclusion, tacitly assumed by Doerr, that the same body is responsible for the anaphylactic effects in the different animals: the utmost it permits is that the production of a precipitate with antigen and precipitin in the circulating blood of a guineapig is followed by a train of symptoms like anaphylaxis.

The gap between the two cases is lessened by an experiment which I may briefly record.

Rabbit young 1050g received 8cc normal rabbit serum followed in $\frac{1}{2}$ hour by 5 cc. Horse Serum, no symptoms: Three weeks later bled 2 cc. then injected intravenous with 5 cc. Horse Serum died in 7 minutes in typical fashion: its serum contained only slight precipitin.

This animal, then, approaches somewhat the guineapig in being sensitive without marked precipitin.

Cell processes probably occur both in the rabbit and guineapig which in their effect on the function of the cell do not differ from those in which as in precipitin animals the nature of the process can be guessed at, (for example Pick and Yamanouchi (75.) have produced in rabbits anaphylactic symptoms with homologous serum. Rabbits sensitised to horse-serum received intravenous the serum of rabbits treated with dog's serum.)

serum in vitro prevent the horse-serum in the mixture from producing anaphylaxis. THE CHEMISTRY OF ANAPHYLAXIS. The

conditions are quite peculiar, it is apparent, and indicate again that the factor is in the body cells

Biochemical studies have so far failed to elucidate these. The papers on the Chemistry of Anaphylaxis which have hitherto appeared are contradictory. Gay and Adler (36) found that the englobulin fraction of horse-serum was highly sensitising but very little toxic. Bruynoghe (18) controverts this absolutely and finds that all fractions of the protein are capable of sensitising and assaulting. Wells (98) shows that the heat effect on the proteins (cf. Besredka p.) by which the assaulting capacity is diminished is directly due to coagulation. With ovomucoid which is not coagulable by boiling both sensitising and assaulting properties persist.

No treatment of horse-serum short of complete denaturation prevents the sensitising action ; the assaulting power persists in reverse proportion to the completeness of this. Prolonged peptic digestion (Wells (loc.cit.)) impairs the assaulting but not the sensitising property. Prolonged contact of the horse-serum with the organs of sensitive animals does not affect its toxicity on using it as an assaulting dose, - i.e. nothing is abstracted from it.

Nor does mixture with sensitive guineapig's serum/

serum in vitro prevent the horse-serum in the mixture from producing death in the sensitive animal. The conditions are quite peculiar, it is apparent, and indicate again that the factor is in the body cells and not in either body fluids or antigen.

The other biochemical data have been already mentioned in the descriptions.

THE SERUM SICKNESS IN MAN.

The importance of experimental investigation of anaphylaxis depends largely on the deleterious effects which horse-serum is capable of producing in man by parenteral administration.

(The appearance of **peculiar** exanthemata in patients treated with antitoxic horse-serum was early remarked upon, - (for the literature of these early observations see v. Pirquet and Shick (70)). Johannessen (45) in 1895 proved that normal horse-serum was equally capable of inducing these. Von Pirquet and Shick () however, were the first to appreciate the nature of the condition.)

The serum sickness appears in 8-10 % of cases after a primary dose of horse-serum (Hartung (40)) the percentage of cases and the severity of the symptoms being roughly proportional to the size of the dose. An incubation period of 8-12 days is characteristic. Thereafter fever, urticaria, swelling of lymphatic glands and joints, oedema, (general, evidenced/

evidenced by increased weight the food supply remaining constant), and slight albuminuria. There may be depression with vomiting and loss of appetite.

On reinjection, depending on the lapse of time since the previous dose, two phenomena may appear. There may be an "immediate reaction" characterised by symptoms within a few hours, - violent urticaria, asthma, prostration, vomiting - passing off within one or two days or there may be an 'accelerated reaction' rather more severe than the primary and occurring 5-7 days after injection instead of 8-12. The size of the primary dose according to Currie(106) does not influence these reinjection phenomena, Goodall, however, (107) thinks a large primary dose increases the likelihood of severe reaction appearing on reinjection. All are agreed that the lapse of time between successive administrations is important; where this is short - under six months, severe immediate reactions occur, over six months these are rare but the accelerated reaction appears. Both may occur on a second injection $1\frac{1}{2}$ -6 months after. The percentage of cases of serum sickness with a second injection is much higher than with the primary, reaching as much as 90 %.

THE CAUSE.

In a recent paper (31) the same observer has demonstrated a temporary diminution in complement during V. Pirquet and Shick (loc. cit) investigated the precipitin contents of the serum in several cases. Simultaneous with the appearance of the symptoms on a primary or repeated dose they found a critical appearance of the precipitin. They could find, however, no definite parallelism between the degree of precipitin formation and the severity of the symptoms. In a later paper (71) they express their conclusions in the general form that the symptoms depend on the union of the horse-serum with an antibody formed by the individual; in consequence there appears a poison either liberated by digestion or linked up by the antibody to the cells. The primary sickness is due to the combination of unexcreted serum with the newly formed antibody hence the incubation period. The immediate reaction on reinjection occurs where this antibody is already present in sufficient quantity, the accelerated reaction depends on the fact previously shown by V. Dungern (110) that artificial antibodies after disappearance take several days less time to reappear on repeating the stimulating injection than with the first attempt.

Francioni (30) also has been struck by the remarkable synchronising of precipitin appearance and/

and the symptoms of serum sickness.

In a recent paper (31) the same observer has demonstrated a temporary diminution in complement during the persistence of the symptoms (cf. my experiments p. 26). My experiments on rabbits lead me to think that the precipitin is certainly one of the factors. The injurious effect of the union of precipitin and antigen in the circulation on the endothelial cells has already been elaborated. An individual with definite precipitin in his serum is without doubt a dangerous subject for reinjection with the antigen. On the other hand - and we have been forced to the same conclusion with the rabbit - there is besides a peculiar condition of the cells in the sensitive animal which is also responsible and may in some cases play the chief part (e.g. after a long interval in particular). The character of the symptoms in man is of great interest as lying intermediate between those of the rabbit and those typical of the guineapig. The endothelial cell injury theory explains particularly well the peculiar grouping of the symptoms (which are also with the exception of the exanthem reproduced in a body of the experimental animals).

CONCLUSIONS/

Horse-serum as CONCLUSIONS.

I consider that the 'immediate' reaction on reinjection of Horse Serum in man is the result of the combined effects of intracirculatory union of precipitin and antigen and of the same process in the protoplasm of the cells of the capillary endothelium. Reinjection should therefore not be attempted in individuals with a definite precipitin in their serum.

The primary reaction and the 'accelerated' reaction are milder in character owing to the gradual manner in which the precipitin as it is formed is thrown down by the foreign serum persisting in the circulation. The advantages offered by immune-serum treatment outweigh the disagreeable but not dangerous anaphylactic symptoms in these cases. It is probable that a conjunctival reaction with foreign serum might give valuable evidence as to the sensitiveness of previously injected individuals and the severity of this might become the criterion for administering or with-holding antitoxic sera.

PREVENTIVE MEASURES.

In individuals rendered highly sensitive to Horse/

Horse-serum as the result of previous treatment the use of antitoxin derived from other animals - sheep or ox - seems reasonable where the demand for antitoxic treatment is clamant (recurrent severe diphtheria). The experiments of Lesné and Dreyfus [53] in the rabbit indicate that there are decided limitations to this. They find that treatment with a variety of foreign proteins not only renders the animals sensitive to each of those used but to other proteins - a general protein hypersusceptibility is set up.

No treatment of the antitoxic serum itself has any effect; heating lessens the severity of the symptom also deteriorates the antitoxin.

The use of purified antitoxin globulin fraction of the serum is said to lessen the number of cases of exanthemata in practice but this may simply be due to the smaller quantities required: experimental evidence is against its being less likely to produce symptoms. Administration of Calcium Chloride was found by Netter [61] to diminish the severity of the exanthemata.

The natural way of introducing a foreign protein so as not to produce anaphylaxis is by the alimentary canal: if the efficacy as regards antitoxic action of this mode of administration is confirmed it ought to become the routine in any but urgent cases.

OTHER PRACTICAL APPLICATIONS OF THE
interest as ANAPHYLACTIC PHENOMENON.

are the observations of Bohm [14] and
Porter [78] of the precipitation induced by extracts
of the tubercle bacillus. I. THE TUBERCULIN-REACTION.

The tuberculin used in the ordinary
test does is insufficient to cause intracirculatory
precipitation. First described by Koch (47) this form of
hypersensitiveness is a valuable diagnostic agent,
the tuberculous animal reacts to an extract of the
Tubercle Bacillus and its products (old tuberculin)
by fever constitutional disturbance hyperaemia and
haemorrhages round the tuberculous lesions. The local hypersensitiveness is of interest
from the theoretical standpoint also. Cutaneous
(scarification or inunction) percutaneous (the 'Stitch-
reaction') or conjunctival application in sensitive
animals sets up a strictly local inflammation i.e.,
paralysis of the capillaries with hyperaemia and
oedema. A local sensitisation even may be set up
by the application of tuberculin in normal animals
as has recently been shown by Cohn [105] the locally
sensitised spot (conjunction^{VA}) reacts either to local
application or to subcutaneous injection of tubercu-
lin.

In tuberculin-sensitiveness the cell reac-
tion/

reaction is apparently the chief factor. Of great interest as bringing it into line with serum-anaphylaxis are the observations of Bonome [14] and Porter [76] on the precipitation induced by extracts of the tubercle bacillus in the serum of tuberculous individuals. The tuberculin used in the ordinary test dose is insufficient to cause intracirculatory precipitation and thus set up the ordinary anaphylactic syndrome but may act on sensitive cells in the same fashion and produce the modified symptoms which appear. The extreme activity of tuberculin as a sensitiser puts it in a special class.

Production of an anaphylactic syndrome by passive sensitisation of young rabbits with the serum of tuberculous patients has been employed by Yamanouchi [102]. He finds severe symptoms and death with small doses of tuberculin when to the animals 5 cc. of the tuberculous serum is administered the day before. His experiments have been criticised (Kraus [48]), but he has repeated them with the same results though not when using the serum of tuberculous guineapigs for the passive sensitisation (not at least regularly) [103]. The precipitin production here again may probably be the factor of causation.

II. TYPHOID DIAGNOSIS.

Kraus & Doerr [48] were the first to apply the anaphylactic syndrome to diagnostic purposes by passive sensitisation with suspected serum. Successful results have been recorded in typhoid by injecting after the typhoid patients' serum an emulsion of typhoid bacilli intravenously.

I do not consider the method as likely to be reliable. The specificity of the anaphylactic reaction is not very marked and the use of animals where extreme individual variations in susceptibility occur is certainly less reliable than the ordinary 'in vitro' reactions.

III. CANCER DIAGNOSIS.

Pfeiffer (Gratz) [68] has used the temperature depression of anaphylaxis as a criterion, in experiments on the effects of injection into guineapigs of the serum of cancer patients followed by intraperitoneal injection of extracts of malignant tumours. He records positive results agreeing with/

with the clinical data. Kelling [46] however, has recently shown that this reaction is not specific but may equally well be produced by extracts of hen-embryos. It is probably again a case of the law already laid down that the union in the circulation of bodies which in vitro produce a precipitate, is accompanied by anaphylactic symptoms. (That precipitates appear with combinations of cancer extract and the serum of cancer patients is well known and is the basis of the complement fixing method of diagnosis. Kelling (loc.cit) for example describes it).

IV. RELATIONSHIP TO FOOD IDIOSYNCRASIES &c.

It has already been pointed out that certain of the peculiarly anaphylactic poisons are derived from substances, shell-fish (Richet), strawberries (Clopatt [20]) egg albumen (Heidenhain), which produce on consumption in certain individuals, as is well known, symptoms of gastro-intestinal irritation, urticariae, exanthemata &c.

Experimental work on anaphylactic phenomena produced by feeding is very scanty. My own experiments with Rice-protein were negative. Rosenau & Anderson/

Anderson [84] sensitised guineapigs by prolonged feeding on Horse flesh to the effects of intracardiac injection of Horse Serum.

The only experiments bearing directly on food idiosyncrasy are those of Börnstein [15], not yet confirmed; Börnstein fed rabbits (by a tube) with the lens-substance of ox eyes. After 100 administrations of about 1 lens per day several of the animals died suddenly an hour or so after their usual dose. They had developed precipitin to the lens substance. Kraus, Doerr and Sohma [51] had already shown that lens-substance was capable of provoking anaphylaxis by intravenous injection but the feeding experiment is unique in the literature.

The assumption that food idiosyncracies are cases of (natural or?) acquired anaphylaxis seems likely to throw light on the obscure question of the nature of these.

V. RELATION TO IDIOPATHIC

ASTHMA.

Consideration of the phenomena of acute anaphylaxis/

anaphylaxis in the guineapig at once suggests the extraordinary similarity to the symptoms of idiopathic asthma. Wolff-Eisner [102] had some years before pronounced the opinion that hay-fever is a specific anaphylaxis to the pollen of certain grasses and has treated it by subcutaneous injections of 'pollantin', prepared from the flowers of grass.

His opinion depended entirely on the consideration that individuals have been known to acquire the hay fever habit after prolonged subjection to inhalation of grass pollen.

It is also a piece of empirical knowledge that the effluvia (hairs?) of certain animals set up in certain individuals attacks of asthma which never occur so long as these animals are avoided.

On the mechanism of such effects it is much too early to speak but the importance of closer study of the general problem of anaphylaxis is apparent.

VI. RELATION TO THE PROCESSES IN IN-

FECTION DISEASES.

These have already been discussed in connection with the endotoxin theory of anaphylaxis. No other evidence is in existence to indicate that in the acute/

acute infections an anaphylactic syndrome occurs. The long incubation period alone is suggestive: no other point of similarity is perceptible.

POSTSCRIPTUM.

The chief additions to knowledge which I have been able to make on the subject of anaphylaxis are:

- (1). The correlation of precipitin strength and complement diminution with the severity of anaphylactic symptoms in the rabbit.
- (2). Analysis of the mechanism of the symptoms by graphic records.
- (3). Determination of the altered Respiratory exchange in the Rabbit and Guineapig.
- (4). Discovery of the diminished bactericidal and opsonic power of the anaphylactic rabbit's serum.
- (5). Description of the Histology of the Anaphylactic Rabbit.
- (6). Critical consideration of the data brought forward and the enunciation of a hypothesis.

In addition I have been able to confirm several of the recent discoveries in anaphylaxis of the guineapig and to lay stress on the differences in the/

the two species.

Finally some theoretical and practical applications to the practice of medicine have been suggested. It remains for me to express my deep gratitude to Dr. James Ritchie for generous help and advice during the whole of the research and to Dr. J.P. McGowan for much ungrudging assistance with difficulties both technical and otherwise. To Mr. Richard Muir I am indebted for the coloured reproductions in the second volume.

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